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SUPERCONDUCTIVITY

/7310

Yaw Control Through Jet Spoilers Investigated

40080159 Beijing GUOJI HANGKONG [INTERNATIONAL AVIATION] in Chinese No 5, May 88 pp 42-43

[Article by Wu Cheng [0702 2052]]

[Text] In recent years, aerodynamicists have become increasingly interested in the application of jet streams for aerodynamic control. Some familiar examples include the jet flap, which is a jet stream blown from the trailing edge of the wing; the jet tip, which is a jet stream blown from the wingtip; and a jet stream blown along the wing-span. Currently, some aerodynamicists are also studying devices using jet stream blown perpendicular to the wing surface. The jet spoiler is one of these devices.

The twisted spoilers commonly used on today's aircraft have the disadvantage of producing strong coupling between yaw and roll motions. Therefore, when the spoiler is used for yaw control a considerable roll moment is generated, and the reverse is true; also, the larger the angle of attack, the stronger the coupling effect. The concept of using jet streams to replace actual spoilers was inspired by the effective use of jet streams as wing flaps. In fact, jet control techniques have long been used on supersonic missiles to control roll moment with minimum yaw coupling.

Figure 1 shows a half-span wing model used in a wind tunnel test. The airfoil used for the wing model is NACA 0018 with 0.057 x 0.015 m jet slit on both the upper and lower surfaces; a 0-15,000 Pal centrifugal compressor is used to supply pressurized air for the test. The pressure distribution over the wing is obtained from 192 pressure-measuring points, and the wake flow field is measured by 5 needle probes. Tests were conducted for the case where air was blown from both the upper and lower surfaces and for the case where air was blown only from the upper surface.

The high-speed jet blown from the wing surface acts like an air barrier which blocks the main flow over the wing; it not only produces the effect of a solid spoiler, but also interacts with the main flow to form a pair of vortices rotating in opposite directions. This vortex pair also interacts with the wingtip vortices to form a complicated three-dimensional flow field. Since a higher stagnation pressure is required for blowing jets on both the upper and lower surfaces, it was found that for the same jet momentum coefficient, better results are obtained with jets blown from only

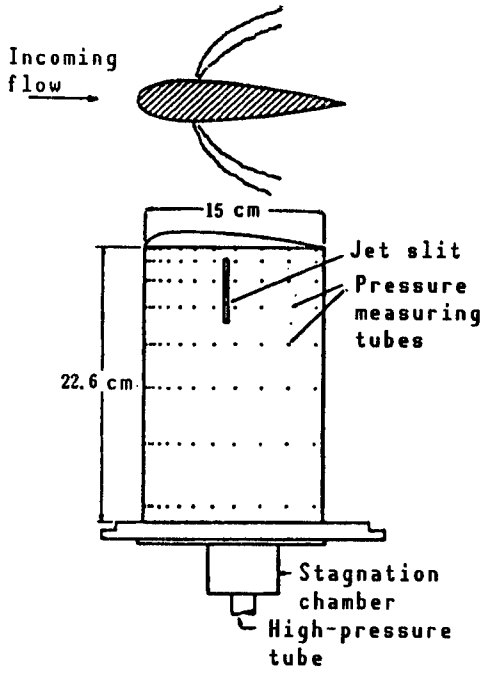

Figure 1. Jet Spoilers and Wing Model

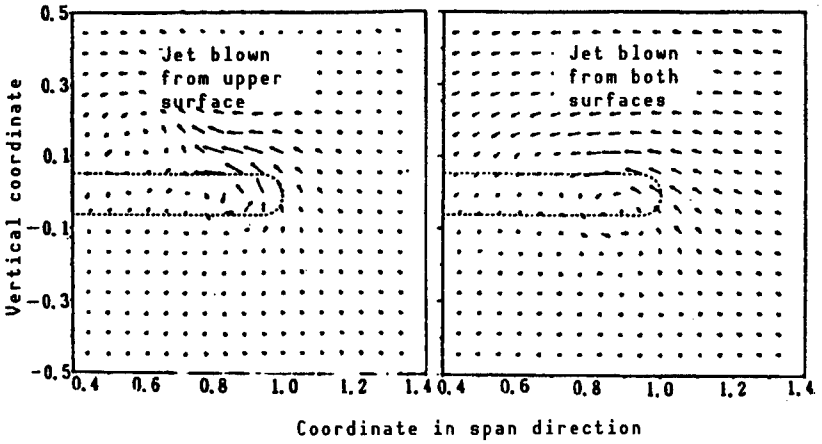

Figure 2. Measured Wake Flow Field Behind the Wing

one side because the formed vortex pairs are denser and more stable. It can be seen from Figure 2 that in the wake flow field one and a half chord-length behind the wing, a vortex pair does exist in the case of the one-sided jet (even though the outboard vortices have merged with the tip vortices), whereas in the case of jets blown from both surfaces, only the tip vortices are visible.

It can be seen from the measured three-dimensional pressure distribution (Figure 3, top) that the effect of jet stream is to raise the forward pressure and lower the rear pressure. This phenomenon can be better

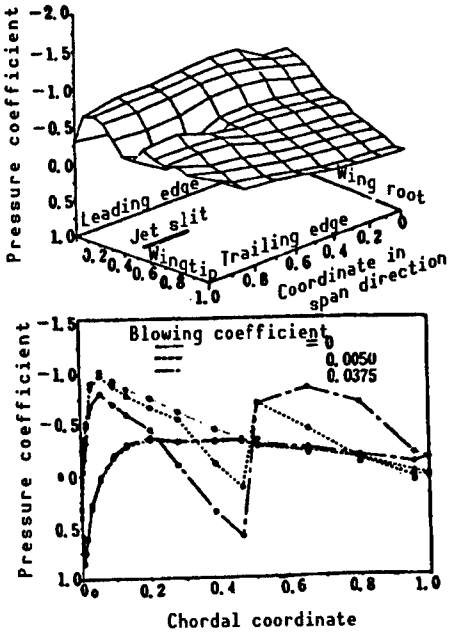

Figure 3. Measured Three-Dimensional and Two-Dimensional (in the chordal direction) Pressure Distributions (one-sided jet)

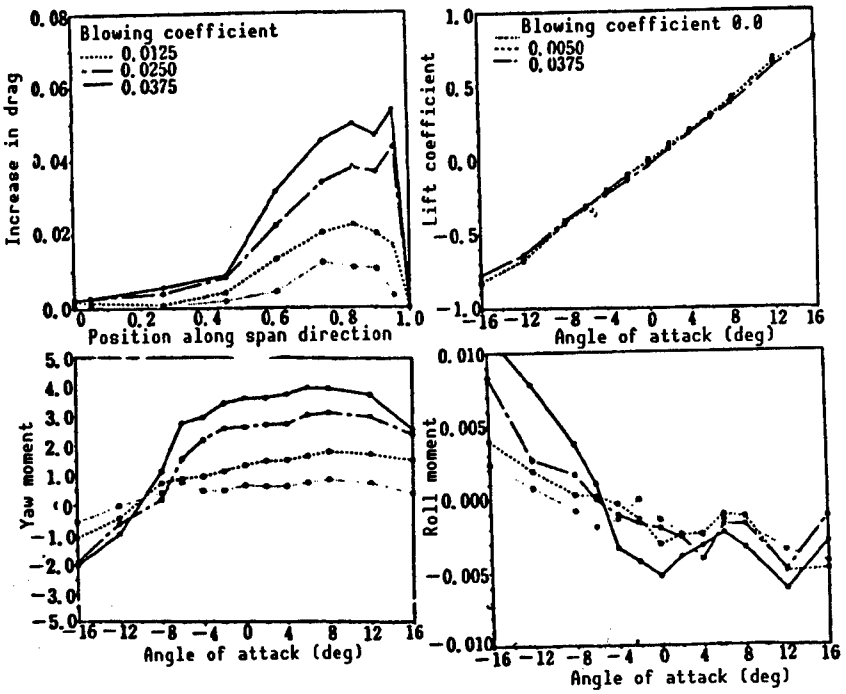

Figure 4. Key Aerodynamic Characteristics

visualized in the chordwise pressure distribution, as shown in Figure 3, bottom figure. This demonstrates that the jet stream separates the main flow from the wing surface, thereby increasing the flow velocity ahead of the jet and decreasing the flow velocity behind the jet, which results in increased drag (Figure 4, upper left). Because the resultant suction point of the total drag produced by the jet is near the wingtip, it can produce an effective yaw moment (Figure 4, lower left). The same figure also shows that for positive angle of attack, the yaw moment is almost independent of angle of attack, and only depends on the jet momentum coefficient. From Figure 4, upper right, it can be seen that this device has little effect on lift; hence the roll moment is much smaller than the haw moment (Figure 4, lower right). This is accomplished by locating the jet slit near the line of maximum wing thickness, thereby partially canceling the normal forces in the vicinity of the jet. Furthermore, because of the inverse pressure distribution, variations in lift are much smaller than drag. Based on simulated calculations, the efficiency of the jet spoiler is higher than that of solid spoilers, and the roll-coupling effect which increases with angle of attack is greatly reduced.

This experiment shows that even with sufficiently high stagnation pressure, the effects of jets blown from both surfaces are not additive; in fact, they cancel one another. Therefore, the one-sided jet design produces uniformly better aerodynamic characteristics.

3012/9365

Integral Method for Low Peclet Number Laminar Pipe-Flow Heat Transfer With Simultaneous Axial Conduction of Fluid, Wall

40090007a Beijing HE KEXUE YU GONGCHENG [CHINESE JOURNAL OF NUCLEAR SCIENCE AND ENGINEERING] in Chinese Vol 8 No 1, Mar 88 pp 1-10

[English abstract of article by Wang Chaoyang [3769 2600 7122], et al., of Zhejiang University]

[Text] It is found that the effects of the combined axial conduction of the fluid and wall on laminar pipe-flow heat transfer in a thermal entrance with a low Pe number are substantial. In this paper, a two-region problem in which the uniform heat flux is imposed in a certain axial position is investigated by the integral method. The analytical solutions obtained are in excellent agreement with Faghri and Sparrow's numerical results. Various possible generalizations of this integral model are also discussed.

9717

Study of Removal of Radioiodine From Nuclear Fuel Reprocessing Off-Gases of Nuclear Power Reactor by HNO_3-$Hg(NO_3)_2$ Scrubbing

40090007b Beijing HE KEXUE YU GONGCHENG [CHINESE JOURNAL OF NUCLEAR SCIENCE AND ENGINEERING] in Chinese Vol 8 No 1, Mar 88 pp 33-38

[English abstract of article by Tang Jingjuan [0781 7234 1227], et al., of Fudan University; Guo Zehong [2654 3419 3163] of Beijing Institute of Engineering]

[Text] The removal of radioiodine from nuclear fuel reprocessing off-gas by HNO_3-$Hg(NO_3)_2$ scrubbing has been studied. The effect of various operating conditions on the removal efficiency of the radioiodine has been investigated. The results show that the removal efficiencies are higher than 99.9 and 99 percent for elemental iodine and methyl iodide, respectively, under appropriate operating conditions. Therefore, employing the HNO_3-$Hg(NO_3)_2$ scrubber as the first equipment to remove radioiodine from nuclear fuel reprocessing off-gases is satisfactory.

9717

Preliminary Crystallography Studies on Binding Between Oxidoreductase and D-Glyceraldehyde-3-Phosphate Dehydrogenase in Muscles of Palinurus Versicolor

40081084a Beijing ZHONGGUO KEXUE [SCIENTIA SINICA, SERIES B] in Chinese No 2, Feb 88 pp 139-143

[Article by Song Shiying [1345 2514 5391], Gao Yigui [7559 5030 6311], Xie Guifu [6200 6311 4395], Zhang Dachuan [1728 1129 1557], Lin Zhengjiong [2651 2397 3518], and Zou Chenglu [6760 2110 7627] of Biophysics Institute, Chinese Academy of Sciences, Beijing]

[Abstract] In the paper, three kinds of crystals of D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in muscles of Palinurus versicolor are cultivated: apo-GAPDH, apo-CM-GAPDH and enzyme crystals containing four fluorescence oxidoreductase NAD derivatives for four crystals. By an X-ray diffraction technique, the space group and crystal cell parameters of the above-mentioned crystals are determined. As indicated in the determination results, these crystals and the corresponding holo-GAPDH crystals saturated with NAD^+ are isomorphs; as suggested with satisfactory isomorphism, the binding between glyceraldehyde phosphate dehydrogenase (from the particular species as mentioned) and oxidoreductase probably does not lead to marked variations in the structural image of relative motion in the structure domain, such as the case with Bacillus stearothermophillus enzyme.

As revealed in the authors' investigation, after removing NAD^+ from holo-GAPDH, the structural stability is reduced; tolerance of X-ray radiation is decreased; and some diffraction intensities change. These facts illustrate that the existence of NAD^+ may have an effect on the structural image of enzyme molecules. The most apparent variation in diffraction intensities of apo- and holo-GAPDH occurs in the hko diffraction spectrum, but not in hol and ohl diffraction spectra. It will be very interesting to learn if this result is associated with the following fact: based on the known orientation of enzyme molecules in these C2 crystal cells, the crystallography c axis is close to the molecule R axis, and the binding sites of NAD^+ are directly linked in pairs through the R axis.

Three figures show an enzyme crystal of apo-GAPDH, as well as photographs of apo-, holo-GAPDH, and photographs at hko layer of apo-, holo- and holo-CM-GAPDH. Two tables list data on the preparation of apo-GAPDH, apo-CM-GAPDH, and holo-Irr-GAPDH after removing two NAD^+; as well as crystal cell parameters and NAD^+ content in six crystal types (apo-, apo-CM, Irr-, holo-, holo-CM and holo-Irr-GAPDH) of P. versicolor GAPDH enzymes. The research was supported by China's State Natural Science Fund. References: 23, 21 in English and 2 in Chinese.

The first draft of the paper was received on 2 February 1987; its revised version was received for publication on 21 May 1987.

10424/9604

Study on Fundamental Characteristics of Spiroplasma in Apis Mellifera. L.

40081084b Beijing ZHONGGUO KEXUE [SCIENTIA SINICA, SERIES B] in Chinese No 2, Feb 88 pp 149-154

[Article by Chen Yongxuan [7115 3057 5503], Xue Baodi [5641 1405 1229] and Guo Yonghong [6753 3057 4767] of Plant Protection Department, Nanjing Agricultural University]

[Abstract] For the first time in China, a spiroplasma isolate CH-1 was obtained from bees by using R-2 culture medium; the isolate has the morphologic structure and mobility of typical spiroplasma. The diameter of CH-1 is 0.17 μm; its length varies with different growth periods. On a solid R-2 culture medium, a fried-egg-shaped or circular colony is formed that has a diameter between 75 and 210 μm. The range of the growth temperature for CH-1 is between 25 and 37°C, with the optimal environment of best growth at pH 7.0 and osmotic pressure at 0.25 mol/L sucrose concentration. Under optimal growth conditions, the growth peak arrives at 48 hours. CH-1 growth requires blood serum, but is inhibited with digitonin. Clucose, fructose, maltose, sugar from seaweed and arginine can be utilized, but not urea. Gelatin cannot be hydrolyzed with CH-1, which cannot reduce methyl blue.

According to the characteristics of CH-1 isolated from spiroplasmas of Apis mellifera. L. (bee) in conforming to the classification standards stipulated by International Systematic Bacteriology Commission, the isolate CH-1 is classified with Spiroplasmataccae, Mollicutes. The biological characteristics of CH-1 are basically the same as S. melliferum and As-576 of bee Spiroplasma; in addition, there are close serological reactions with As-576 (to be reported in another paper). Therefore, in the authors' view, CH-1 should be classified in Subgroup I-2 of Serogroup I in Spiroplasma serological classification. However, they are not completely identical as to the optimal growth osmotic pressure, and the utilization of arginine and mannose as between CH-1 and As-576; they may be two different members in the same serosubgroup.

Six figures show CH-1 colonies, CH-1 and CH-1 growth curves in R-2 culture medium, as well as the growth effect on CH-1 caused by temperature, by osmotic pressure and by the pH of the culture medium. Three tables list data on the growth increments of CH-1 in R-2 culture medium with and without blood serium, growth inhibition imposed by digitanin on CH-1, and CH-1 utilizability for different varieties of sugar. References: 13 in English.

The first draft of the paper was received on 11 March 1987; the revised draft was received for publication on 21 June 1987.

10424/9604

Functions of In-Vitro Transcription of L, NS and N Proteins in Wheat Rosette Stunt Virus

40081084c Beijing ZHONGGUO KEXUE [SCIENTIA SINICA, SERIES B] in Chinese No 2, Feb 88 pp 155-161

[Article by Sun Wei [1327 0251], Gong Zuqin [7895 4371 1053] and Cao Tianqin [2580 1131 2953] of Shanghai Biochemistry Institute, Chinese Academy of Sciences]

[Abstract] The paper reports on the stagewise depolymerization of nucleocapsids of wheat rosette stunt virus (WRSV-NP). By ultracentrifuging with glycerin as cushion, four virus constituents can be obtained: L protein, NS-N-RNA complexes, NS protein and N-RNA complexes. After individual determinations, all these constituents failed to mainfest the activity of RNA polymerase. It was discovered after conducting different recombinations that in-vitro transcription can proceed only with simultaneous existence of L, NS and N-RNA complexes. Moreover, experiments reveal that the binding forces of these three protein varieties with WRSV-RNA are in the following order: N>>NS>L. Based on the result, the authors predicted the following: L and NS proteins can be incorporated into RNA polymerase complexes; N protein (closely binding with virus RNA) may have the function of maintaining activity of genic templates.

In one paper [(11 in references] coauthored by Gong Zuxun, enzyme processing phenomenon of WRSV N protein was reported. In polypropene acylamine gel electrophoresis, N protein is manifested as two stripes as shown in one of text figures; these two stripes are called by authors as N1 and N2 proteins with molecular weights of 46,000 and 44,000 respectively. As to what roles in the replication and transcription of WRSV are played by N protein and its enzyme processing phenomenon, this will be extensively studied by the authors after solving the problem of isolating N1 and N2 proteins. As established by the authors, the in-vitro transcription system will furnish more information in making available a simple experimental model for adjustment and control of WRSV in-vitro replication and transcription. These studies are underway.

Five figures show protein and nucleic acid compositions, as well as functions of in-vitro transcriptions of WRSV-RNA by L, NS and N proteins. One table lists data of WRSV-RNA in-vitro transcription of L, NS and N proteins. The study was supported by China's State Natural Science Fund. References: 11, 6 in English and 5 in Chinese.

The authors are grateful to Chen Kejian [7115 0344 0256] and Shi Yangsheng [1597 0111 0524] of Liaocheng Prefecture Agricultural Bureau in Shandong Province for providing WSRV specimens, and to colleague Peng Baozhen [1756 1405 3791] of the author's institute for developing and enlarging photographs. First draft of the paper was received on 21 March 1987; the revised draft was received for publication on 27 June 1987.

10424/9604

Reproduction Enzyme of Mouse Ascites Liver Cancer Cells, and Relationship Between Variation of Chromosome Structural Image of Diversified Enzyme Genes and the Enzyme Genic Expression

40081084d Beijing ZHONGGUO KEXUE [SCIENTIA SINICA, SERIES B] in Chinese No 2, Feb 88 pp 162-166

[Article by Li Shi'e [2621 1102 6948], Ma Enling [7456 1869 7117] and Wu Shijun [0702 4258 0689] of Fundamental Medicine Department, China Xiehe Medical University; and Fundamental Medicine Institute, Chinese Academy of Medical Sciences, Beijing]

[Abstract] The activity of CPS, of mouse ascites liver cancer cells is much lower than for normal liver cells; however, the activity of ACT increased by about 13 times that of normal liver. As proved with the hybridization of RNA-cDNA spots, the level of CPS_1 is very low in liver cancer cells; however, the level of ACTmRNA is increased over that of normal liver cells. As shown by the results of the inhibitory digestion of cell nuclei in a normal liver and in a cancerous liver by micrococcus nuclease (MCN), glucose agar gel electrophoresis, and hybridization of DNA-cDNA, the actively expressed CPS_1 genes are distributed on nucleoli of loosely structured monomers, dimers and trimers; however, the relatively less expressed ACT genes are distributed on relatively compact oligomer nucleoli. The situation of ascites liver cancer cells is just the opposite. The expression of CPS_1 genes is very low, mainly distributed on oligomer nucleoli, but the expression of ACT genes is intensified, mainly distributed on monomer nucleoli. These results amplify the following facts: there is a close relationship between the interdependent variations in expression of diversified enzyme CPS_1 genes and of reproduction enzyme AST genes in liver cancer cells, and the interdependent variations of the nucleolar structural image of these two kinds of genes.

Two figures show the expression of CPS_1 and CAD mRNA in normal mouse liver and mouse ascites liver cancer cells, the distribution of CPS_1 and CAD DNA sequences in nucleoli of normal mouse liver and mouse ascites liver cancer cell after partial breakdown by MCN. One table lists data on CPS_1 and ACT activities in normal mouse liver and mouse ascites liver cancer cells. References: 35; 26 in English and 9 in Chinese.

The authors are grateful to Li Yinxiong [2621 1438 7160] and Yu Yue [0060 6460] for their determination of enzyme activities. The first draft of the paper was received on 2 August 1986; the revised draft was received for publication on 3 December 1986.

Study of Duck Liver Cancer Genes--MHT (RAF) and HA-RAS

40081084e Beijing ZHONGGUO KEXUE [SCIENTIA SINICA, SERIES B] in Chinese No 2, Feb 88 pp 167-173

[Article by Tan Hong [6151 4767], Liu Chaoting [0491 6389 0080], Hong Weinuo [3163 0251 1226], Tang Guoying [3282 0948 5391], Jiang Huiqiu [5592 1920 4428], Chen Yuanqing [7115 3220 0615] and Gu Jianren [7357 0256 0086] of Shanghai Institute of Oncology; and Zhang Suyin [1728 4790 5255] and Lu Zhixiang [7120 1807 4382] of Shanghai Institute of Materia Medica, Chinese Academy of Sciences]

[Abstract] Liver cancer is one of most commonly seen malignant tumors in China. In areas of high incidence of liver cancer in China, there is a correlation between the high incidence of human and duck primitive liver cancers. As reported by Chang in 1973, observations of multifactor pathogenesis of human liver cancer cases and animal models of duck liver cancer are consistent。

For the first time, the paper reports on the invert activities of duck liver cancer DNA and adjacent tissue (of cancer) DNA on NIH 3T3 and RAT-1 cells. From an analysis of DNA of invert cell specimens, it was proven that the sequence of mht (mil or raf) and C-Ha-ras exists in all invert cell specimens of DNA in duck liver cancer cells and cells in tissue adjacent to cancer. This result significantly illuminates the molecular mechanism of agents inducing duck cancer: hepatitis B virus and chemical causative agents. Since research on duck liver cancer is helpful to the study of human liver cancer, the authors will establish a model of duck liver cancer in order to explore the potential molecular mechanism.

Seven figures show cells of invert foci using NIH 3T3 cells, 30 days after inoculation with soft agar in NIH 3T3 invert cell specimen, the external appearance of a mouse 40 days after being inoculated with TR40K of RAT-1 invert cell specimen, a positive contrast between 3T3 and an invert cell specimen DNA, on the one hand, and normal duck liver DNA, on the other; hybridization bands of normal duck liver and invert cell specimens, and contrast between duck liver RAT-1 DNA and RAT-1 invert cell specimen DNA after EcoRI zymolysis using a V-mht probe. References: 7 in English

The authors are grateful to Dr Thomas Shih of NCI (National Cancer Institute) for providing V-ki-ras clone (HiHi-3), Dr T. Mak of the Canadian Ontario Cancer Research Institute for providing N-ras (Wigler), and Dr Nancy Kan of NCI for providing V-mht. The research was jointly supported by China's State Natural Science Fund, the World Health Organization, and the Western Pacific Regional Organization. First draft of the paper was received on 8 November 1986; the revised draft was received for publication on 2 May 1987.

10424/9604

RFLP of Phenylketonuria Gene Studied

40081081 Beijing ZHONGGUO KEXUE [SCIENTIA SINICA SERIES B] in Chinese No 3, Mar 88 pp 297-302

[Article by Zeng Yitao [2582 3300 3325], Huang Shuzheng [7806 3219 1608], Chen Meiyu [7115 5019 6877], Zhang Meilan [1728 5019 5695], and Ren Zhaorui [0117 0340 3843], Genetics Section, Shanghai Municipal Pediatrics Hospital; Xu Yonghua Who [1776 3057 5478], and Peng Sufen Fen [1756 4790 5358], Shanghai Cytobiology Research Institute, Chinese Academy of Sciences; and Hu Liuqing [5170 3177 3237], Cytobiology Department, Baylor College of Medicine, U.S.A.: "Study of Restriction Fragment Length of Polymorphism of the Phenylketonuria Gene in Chinese"]

[Text] Abstract: This article reports the use of human phenylalanine hydroxylase (PAH) cDNA as a hybrid probe in the analysis of the polymorphic locations of restriction endonuclease in the PAH gene of both 80 normal Chinese people and 28 Chinese people suffering from phenylketonuria (PKU) at Bgl II 3.6kb/1.7 kb, EcoRI 17kb/11kb, EcoRV 30kb/25kb, Hind III 4.2kb/4.0kb, Msp Ia 23kb/19kb, Msp Ib 4.0kb/2.2kb, Pvu IIa 19kb/6.0kb, and Pvu IIb 11.5kb/9.1kb. The frequency of occurrence of restriction fragment length polymorphism (RFLP) in the PAH gene of normal Chinese was 0.13, 0.83, 0.77, 0.81, 0.12, 0.04, 0.70, and 0.10 respectively. The frequency of occurrence of the aforementioned RFLP when the PAH gene was lacking was 0.12, 0.93, 0.89, 0.81, 0.04, 0, 0.69, and 0.04 respectively. This demonstrates a substantial difference between Chinese and the white race in PAH gene RFLP, and that assay of PAH gene RFLP in Chinese may be used for prenatal diagnosis of PKU.

Phenylketonuria (PKU) is a serious amino acid metabolism disorder of genetic origin in which sufferers are unable to convert phenylalanine hydroxylase into tryosine because of a lack of phenylalanine (PAH, EC 1.14. 16.1) in their livers. Consequently, phenylalanine hydroxylase builds up in their bodies, is metabolized into phenylpyruvic acid, and is eliminated in the urine. Unless this condition is treated immediately following birth, serious retardation of mental development will occur.[1,2] The frequency of occurrence of this disorder in Chinese is approximately one per 16,000[3,4]. Approximately one out of every 65 people carries a defective PAH gene.

The PAH gene is found in human chromosome number 12 ($12q^{22} - 12q^{24\ 1}$).[5] The nucleotide sequence of a PAH cDNA clone[6] recently isolated from a human liver cDNA library was recently defined,[7] and research was undertaken on the RFLP of the PAH gene in members of the white race,[8] making prenatal PKU diagnosis and heterozygote detection possible.[9] This article reports the use of human PAH cDNA as a probe to analyze RFLP of the PAH gene in both normal Chinese and those afflicted with PKU.

1. Materials and Methods

1. Subjects of the Analysis

Thirty-three blood samples from normal people were taken from workers in the Shanghai Municipal Pediatrics Hospital, and 47 samples were taken from the umbilical cord blood of newborns at the Changning District Gynecological Hospital in Shanghai. Twenty victims of PKU and their parents were outpatients for the hospital's Genetics Section. PAH in the blood serum of PKU victims was higher than 20 mg/dl, a classic indicator of PKU.

2. Test Methods

(1) DNA Extraction: DNA was extracted from peripheral white blood cells using the laboratory's conventional method.[10]

(2) PAH Gene RFLP Analysis: Enzymolysis was done by using 10 units of restriction endonuclease on each of 5 μg of DNA, Bgl II, EcoRI, EcoRV, Hind III, Msp I or Pvu II. The DNA segments from the enzymolysis were isolated through agarose electrophoresis, and Southern print transfer, hybridizing and autoradiography was done following the methods described in a previous article.[10]

(3) PAH cDNA Probe Preparation: After recombinant plasmid ph PAH 247[8] was digested by EcoRI, a 2.4kb long PAH cDNA segment isolated through agarose electrophoresis was subjected to the notch translation method to prepare a ^{32}P-marked PAH cDNA probe.[10]

2. Results

1. PAH Gene RFLP

The following restriction enzymes were used in the enzymolosis of Chinese DNA specimens, the polymorphic DNA segments that appeared on the enzyme cutting atlas being as follows. (See Figure 1):

Bgl II produced 3.6kb or 1.7kb polymorphic PAH DNA segments.

EcoR I produced 17kb or 11kb polymorphic PAH DNA segments.

EcoR V produced 30kb or 25kb polymorphic PAH DNA segments.

Hind III produced 4.2kb or 4.0kb polymorphic PAH DNA segments.

Msp I produced 23kb or 19kb + 4kb polymorphiic PAH DNA segments. In addition, if a polymorphic enzyme point existed on a 4.0kb DNA segment, it was cut further to produce a 2.2 kb and a 1.8 kb PAH DNA zone.

Pvu II produced 19kb or 6.0kb; 11.5kb or 9.1kb polymorphic PAH DNA segments.

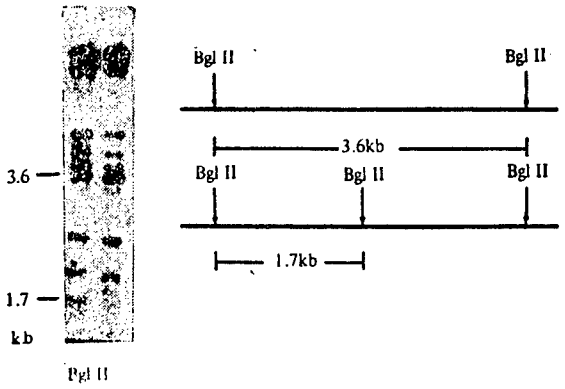

Figure 1(a) Bgl II RFLP of a Chinese PAH Gene (Detected by Human PAH cDNA Probe)

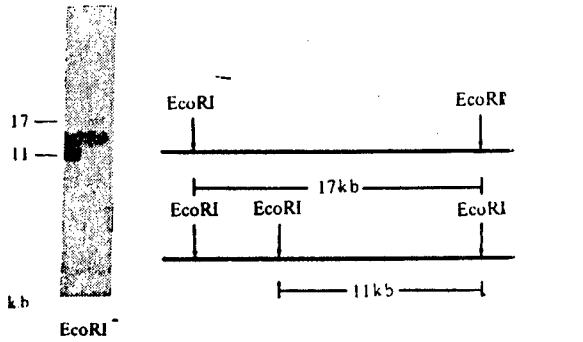

Figure 1(b) Eco RI RFLP of a Chinese PAH Gene (Detected by Human PAH cDNA Probe)

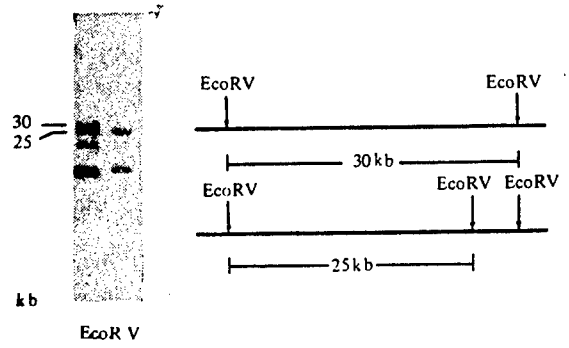

Figure 1(c) Eco RV RFLP of a Chinese PAH Gene (Detected by Human PAH cDNA Probe)

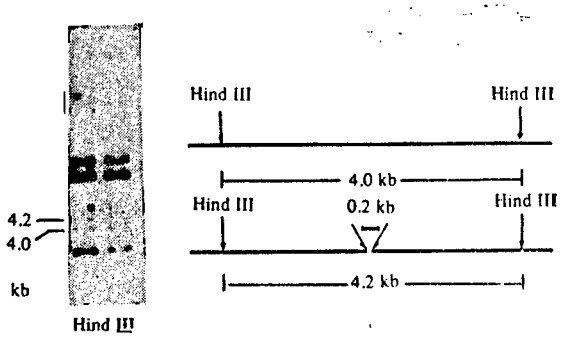

Figure 1(d) Hind III RFLP of a Chinese PAH Gene (Detected by Human PAH cDNA Probe)

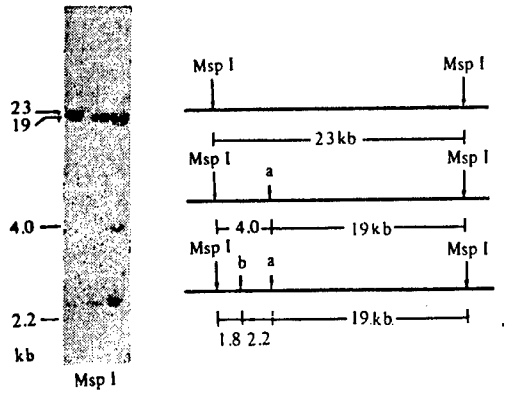

Figure 1(e) Msp I RFLP of a Chinese PAH Gene (Detected by Human PAH cDNA Probe)

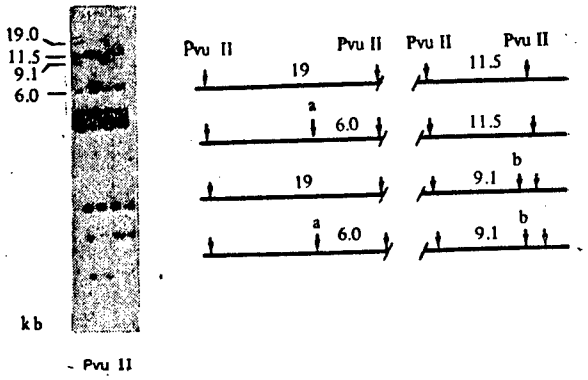

Figure 1(f) Pvu II RFLP of a Chinese PAH Gene (Detected by Human PAH cDNA Probe)

2. PAH Gene RFLP Occurrence Rate

Comparison of occurrence rates for PAH gene RFLP detected in normal Chinese and Chinese sufferers from PKU with occurrence rates in members of the white race is shown in Table 1.

3. For more than one of the eight different kinds of PAH gene RFLP among the 80 normal Chinese analyzed in this research, the restriction endoclase location had a heterozygote. That is a total of 65 cases (approximately 80 percent). In 15 cases (approximately 20 percent), it had a homozygote with no heterozygote present.

Among the 28 PKU families analyzed for this article, either one or both parents had heterozygotes at the restriction fragment length polymorphism of the PAH gene; thus it was possible to use RFLP chain analysis to make a prenatal diagnosis or to detect heterozygotes in 22 family lines. (See Figures 2(a) and (b)).

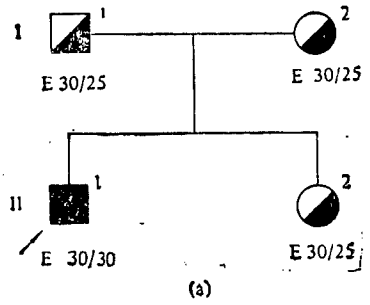

Figure 2(a) PKU Lineage 1 (PKU Heterozygote Detection Using Restriction Endoclase EcoRV(E))

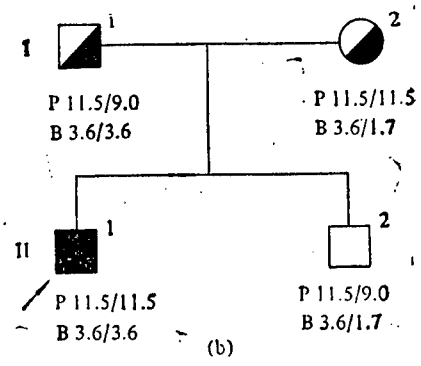

Figure 2(b) PKU Lineage 2 (PKU Heterozygote Detection Using Restriction Endoclase EcoRV(E))

Male Heterozygote
Female Heterozygote
Male PKU Victim
Normal Male Proband

Figures beside the endoclase denote the size (kb) of the PAH gene specific DNA segment.

Table 1. Occurrence Rate for PAH Gene Restriction Fragment Length Polymorphism

Restriction Endoclase	DNA Segment (kb)	Chinese* Normal Chromosome		Chinese PKU Chromosome		White Race** Normal Chromosome	
		N	F	N***	F***	N	F
Bgl II	3.6(-)	113	0.87	46	0.88	19	0.59
	1.7(+)	17	0.13	6	0.12	13	0.41
	Total	130		52		32	
EcoR I	17(-)	26	0.17	4	0.07	19	0.59
	11(+)	130	0.83	52	0.93	13	0.41
	Total	156		56		32	
EcoR V	30(-)	36	0.23	6	0.11	17	0.47
	25(+)	120	0.77	50	0.89	19	0.53
	Total	156		56		36	
Hind III	4.2(-)	30	0.19	10	0.19	22	0.61
	4.0(+)	124	0.81	42	0.81	14	0.39
	Total	154		52		36	
Msp Ia	23(-)	136	0.88	54	0.96	12	0.38
	19(+)	18	0.12	2	0.04	20	0.62
	Total	154		56		32	
Msp Ib	4.0(-)	148	0.96	56	1.00	22	0.69
	2.2(+)	6	0.04	0	0	10	0.31
	Total	154		56		32	
Pvu IIa	19(-)	47	0.30	17	0.31	16	0.44
	6.0(+)	109	0.70	37	0.69	20	0.56
	Total	156		54		36	
Pvu IIb	11.5(-)	140	0.90	52	0.96	25	0.69
	9.1(+)	16	0.10	2	0.04	11	0.31
	Total	156		54		36	

*Normal hormones for Chinese included normal hormones from both normal people and from the parents from some PKU victims; the PKU chromosome count includes only chromosome from PKU victims, and does not include chromosomes from parents carrying a defective PAH gene.

**Data on members of the white race was taken from Lidsky et al.[9]

***N denotes the number of chromosomes analyzed; F denotes the occurrence rate.

3. Discussion

Restriction fragment length polymorphism is caused by gene neutral mutation. This kind of neutral mutated gene's base substitution does not effect gene functioning in any way; however, because of the base substitution, the cutting point of the restriction endonuclease is changed. We can draw on the proper restriction endonuclease and gene probe to detect it. It has been estimated that one out of every 100 bases in human genes has a base substitution that gives rise to restriction fragment length polymorphism.[11] Gene restriction fragment length polymorphism frequently exists in racial differences; thus thorough understanding of data about the gene polymorphism of all races can provide a reliable basis for anthropological study on a molecular level of mankind's origins, and the movements and evolution of peoples.

The PAH gene probe used in the work covered by this article included the entire human PAH cDNA sequence; therefore, it was possible to detect accurately PAH gene polymorphism.[7,8] One can see from Table 1 that the polymorphism rate of occurrence at eight locations for the aforementioned six different restriction enzymes of the PAH gene in Chinese is different than for members of the white race.[8] In Chinese, the PKU occurrence rate is approximately one per 16,000, which is lower than the one per 10,000 for members of the white race.[12] The authors recently further tested the PAH gene polymorphic haplotypes, finding four haplotypes to be different than in members of the white race. This suggests the possible existence of a certain difference in the molecular basis for PKU of the two races.

PAH exists in the liver, and the metabolism of phenylalanine hydroxylase into phenylpyruvic acid also takes places in the liver. Neither human blood serum nor skin fibryocytes contains PAH; thus the prenatal diagnosis ofPKU cannot rely on fetal blood, fetal cell, or amniotic fluid gene products or products of metabolism for detection. However, if the polymorphic restriction endonuclease cutting point and the chain genetic relationship of the deficient gene of every member of a family line is analyzed, polymorphic locations can serve as "genetic markers" for accurate prenatal diagnosis of genetic illnesses. Recently we used PAH gene analysis to complete the first prenatal diagnosis of PKU in a Chinese.[13] The analytical results of the work covered by this article show with regard to the eight locations of polymorphism in the PAH gene that in approximately 80 percent of Chinese there is a heterozygote. When the heterozygotes at these polymorphic locations are married to each other, or when they are married to a homozygote, if a PAH deficient gene exists, prenatal diagnosis for PKU and heterozygote detection may be done using the RFLP chain analysis method.

Among the PKU family lines surveyed in this article, either one or both parents in 79 percent of all the families had a PAH gene polymorphic location heterozygote; consequently, it was possible to make a prenatal diagnosis of PKU and to detect heterozygotes. Figure 2(a) shows the PAH gene polymorphic genetic relationship in a PKU family line in which a heterozygote assay was done. In this family line, both parents had

polymorphic heterozygotes (30kb/25kb) at the EcoRV cutting point, but in the PKU victims, there were no polymorphic homozygotes (30kb/30/kb) at the EcoRV cutting point. This shows the chromosomes of the parents of this family line did not have EcoRV polymorphic cutting point chromosomes (30kb/); they carried a deficient PAH gene. Two months after this family's second child was born, a gene diagnosis was requested, which found the simultaneous existence on the EcoRV zymogram of a PAH gene specific zone (30kb/25kb) at 30 kb and 25 kb. This showed that in her cells, only one chromosome (30kb/) carried a deficient PAH gene; thus it was found that this infant had a PKU heterozygote. Figure 2(b) shows the genetic relationship of the PAH gene in yet another family line in which the author made a prenatal diagnosis of PKU. In this family, the father (I-1) had a Pvu IIb polymorphic location heterozygote (11.5kb/9.1kb), and the victim (II-1) had no such polymorphism (11.5kb/11.5kb). Clearly the father's Pvu IIb polymorphic location chromosome (9.0kb) carried a normal PAH gene. Furthermore, the mother (I-2) in this family had a heterozygous Bgl II polymorphic location (3.6kb/1.7kb), and the victim had no homozygote (3.6kb/3.6kb) at the Bgl II polymorphic location, showing that the mother's Bgl II polymorphic location chromosome (1.7kb/) was a normal chromosome that did not carry a defective PAH gene. As a result of the chain relationship of the restriction fragment length polymorphism and the PAH gene in this family line, we made a prenatal diagnosis of the embryo during the wife's second pregnancy. During the ninth week of pregnancy, we extracted villus DNA from fetal villi. We identified the fetus' (II-2) PAH gene's restriction fragment length polymorphism to be Pvu IIb 11.5 kb/9.0 kb, and Bgl II 3.6 kb/1.7 kb, showing that the fetus had inherited normal PAH genes from its parents; thus the fetus was diagnosed as being normal. The author has completed prenatal diagnosis of 10 PKU high risk fetuses in Shanghai using PAH gene restriction fragment length polymorphism chain analysis.

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9432/9604

Increased Protein, Amino Acid in Nucleoplasmic Wheat Hybrids Studied

40081082a Beijing KEXUE TONGBAO in Chinese Vol 33 No 5, Mar 88 pp 377-381

[Article by Wu Yuwen [0702 6735 2429], Zhang Cuilan [1728 5050 5695], and Zhang Yan [1728 3508], Genetics Institute, Chinese Academy of Sciences, Beijing: "Effects of Allocytoplasm on Wheat Grain Protein and Amino Acid Content"]

[Text] Study of the genetic effects of allocytoplasm on wheat grain protein content has not yet attracted a great deal of attention. Though some researchers have reported the introduction of common wheat (Triticum aestivum) cell nuclei into the cytoplasm of certain genetically related varieties of wheat, thereby increasing the protein and lysine content of wheat grain as a result of the effects of the cytoplasmic or nucleo-cytoplasmic interaction,[1-3] for the most part these were results obtained from single experiments with a single generation of material. In some cases the number of replacement backcrosses of test materials supplied were not sufficient, and some people also noted that under most circumstances, the fairly high protein count of the allocytoplasm line was attributable to the secondary effects of the cytoplasm on variations of a quantitative nature.[4] Much data show that in a proper nucleo-cytoplasmic combination, allocytoplasm has a nuceloplasmic heterosis effect on some agronomic traits. If these effects can be used to improve seed quality, that would, without doubt, be beneficial for the breeding of cereal crops.

From 1984 through 1986, we observed five generations (B_3-B_7) of nucleoplasmic hybrids containing Aegilops squarrosa wheat cytoplasm, and we studied the genetic effects of the cytoplasm on grain protein and amino acid content. We also discussed the correlation between the protein content and several major agronomic traits.

1. Materials and Methods

The allocytoplasm line provided was a single No 11 strain of Aegilops squarrosa wheat (Aegilops squarrosa 011), which became the cytoplasm donor. The nucleus donor was the Kenya variety of ordinary wheat (Triticum aestivum). Young embryo culturing done in 1978 surmounted the lack of affinity between the two, and an intergeneric hybrid was obtained, which was then successively replacement backcrossed to obtain an Aegilops

squarrosa line with a replacement nucleus, (or a (squarrosa)-Kenya)[5] for short). The present experiment obtained five generations, B_3-B_7 of grains of the line in which the nucleus had been replaced, and the Kenya variety grains having the original cytoplasm were used as a control. The material supplied for the experiment was sown in open fields during the spring of 1984, 1985, and 1986 in Beijing. Small plots containing two rows, each row being 2 meters long and having a distance of 35 centimeters between rows were planted three times in a random order. At harvest time, separate strains were selected for examination of main spikes. The mixed materials were threshed and samples were taken at random for assay of the grain protein and analysis of amino acid. Over the 3 year period, the analysis laboratory of the National Resources Comprehensive Survey Committee of the Chinese Academy of Natural Sciences, the Comprehensive Analysis Laboratory of the Chinese Academy of Agricultural Sciences, and the Cereals, Fats, and Oils Chemical Institute of the Ministry of Commerce assayed the grain protein content, and the Comprehensive Analysis Laboratory of the Chinese Academy of Agricultural Sciences assayed the lysine content. The Negative Pressure Laboratory of the Chinese Academy of Science's Genetics Institute assayed the amino acid makeup and content. It also did a quantitative analysis and a t-value testing of data pertaining to several agronomic traits.[6]

Kjeldahl's method was used to assay the seed's protein content, and a Hitachi Model 835-50 completely automatic amino acid analyzer was used to assay the content of 17 different amino acids using the acid hydrolysis method in which 6 mol per liter of HCl was hydrolized at 110 degrees C for 24 hours.

2. Results of Experiment

a. Variations in Seed Protein Content. Results of the assay of grain protein by the three units, which appear in Table 1, show the protein content of the B_3-B_7 generation of the allocytoplasm line, which was in the material analyzed by the Comprehensive Analysis Laboratory of the Chinese Academy of Agricultural Sciences, to have increased by between 2.17 and 3.71 percent over that of the control. It was highest in the B_6 and B_7 generations. Data from the Comprehensive Analysis Laboratory of the Chinese Academy of Agricultural Sciences (1985) showed an increase in seed protein content of from 0.70 to 1.87 percent. As replacement backcrossed generations increased, the protein content increased correspondingly as well, being highest in the B_6 and B_7 generations. Results found by the Cereals, Fats, and Oils Institute of the Ministry of Commerce (1986) further demonstrated this tendency toward increase. Results obtained by the three units over a 3 year period in the assay of seed protein content were virtually identical, with differences only in the extent of increase.

Table 1. Nucleo-cytoplasmic Hybrid (squarrosa)-Kenya Seed Protein Content

Materials	Comprehensive Survey Analysis Laboratory, 1984 Chinese Academy of Sciences		Comprehensive Analysis Lab Chinese Academy of Agricultural Sciences 1985		Cereals, Fats & Oils Chemistry Office, Ministry of Commerce	
	Protein Content Control	Percent of Increase Over Control	Protein Content Control Equivalent %	Percent of Increase Over Control	Protein Content Increase Over Control %	Percent of Increase Over Control
Kenya (ck)	14.53 (100)	—	16.65 (100)	—	15.00 (100)	—
(*squarrosa*)-Kenya B_3	16.70 (114.93)	2.17	17.35 (104.20)	0.70	17.87 (119.13)	2.87
(*squarrosa*)-Kenya B_4	16.70 (114.93)	2.17	17.12 (102.82)	0.47	17.99 (119.93)	2.99
(*squarrosa*)-Kenya B_5	17.04 (117.27)	2.51	17.32 (104.02)	0.67	18.21 (121.40)	3.21
(*squarrosa*)-Kenya B_6	18.24 (125.53)	3.71	18.52 (111.23)	1.87	18.06 (120.40)	3.06
(*squarrosa*)-Kenya B_7	17.94 (123.47)	3.41	18.28 (109.79)	1.63	18.50 (123.33)	3.50
Percent Increase of Each Allocytoplasm Line Generation Over Control	17.32 (119.23)	2.79	17.72 (106.41)	1.07	18.13 (120.84)	3.13

Results of the experiment showed the allocytoplasm line grain protein content of all generations of the (squarrosa)-Kenya to be higher than that of the original cytoplasm Kenya nucleus parent variety, and there was a tendency for the protein content to increase as the number of generations increased. This change was fairly consistent, and could be duplicated.

In the course of the assay by the Chinese Academy of Agricultural Science's analytical laboratory, the renowned high protein wheat variety, Atlas 66, was also added as a control. The seed protein content assayed for the Atlas 66 was 18.13 percent, while it was 18.52 percent for the (squarrosa)-Kenya B_6, and 18.28 for the B_7 generation. Clearly the protein content of the allocytoplasm line provided for the test was exceptionally close to that of the Atlas 66.

2. Differences in the Seed Amino Acid Components. The 17 amino acids assayed in this experiment were as follows: aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, agrinine, proline, and cystine.

The data provided in Table 2 show the following: The content of the 17 different amino acids in the seeds of each line in the B_3-B_7 generations of the (squarrosa)-Kenya is higher than for the same amino acids in the corresponding Kenya nucleus parent variety. Without exception, the total

content of the 17 different amino acids in all lines of the B_3-B_7 (the number of milligrams per 100 milligrams of dried material) was 15.7692, 15.1695, 16.1671, 15.5593, and 16.5039 respectively, for an average of 15.8338 in each line, an amount that markedly exceeds that of the control nucleus parent (12.5767).

Table 2. Comparison of Amino Acid Composition of (squarrosa)-Kenya Allocytoplasm Line Grain

Particulars / Amino Acids	Kenya (ck)		(*squarrosa*)-Kenya B_3		(*squarrosa*)-Kenya B_4		(*squarrosa*)-KenyaB$_5$		(*squarrosa*)-Kenya B_6		(*squarrosa*)-Kenya B_7		
	mg / 100mg dry material	%	mg / 100mg dry material	%	mg / 100mg dry material	%	mg / 100mg dry material	%	mg / 100mg dry material	%	mg / 100mg dry material	%	mg / 100mg dry material	%
Aspartic Acid	0.6110	100	0.7648	125.17	0.6770	110.80	0.7184	117.58	0.6883	112.65	0.7300	119.48	0.7157	117.14
Threonine	0.3475	100	0.4317	124.23	0.3901	112.26	0.4308	123.97	0.4025	115.83	0.4354	125.29	0.4181	120.32
Serine	0.5379	100	0.6808	126.57	0.6340	117.87	0.6985	129.86	0.6615	122.98	0.7185	133.58	0.6787	126.18
Glutamic Acid	4.1969	100	5.2665	125.49	5.1038	121.61	5.5016	131.09	5.2322	124.67	5.6852	135.46	5.3579	127.66
Glycine	0.4652	100	0.6109	131.32	0.5747	123.54	0.6224	133.79	0.6001	129.00	0.6288	135.17	0.6073	130.57
Alanine	0.4134	100	0.5303	128.28	0.4947	119.67	0.5365	129.78	0.5116	123.75	0.5407	130.79	0.5227	126.46
Valine	0.5608	100	0.7016	125.11	0.6769	120.70	0.7241	129.12	0.6855	122.24	0.7327	130.65	0.7042	125.57
Methionine	0.2186	100	0.2339	107.00	0.2269	103.80	0.2311	105.72	0.2960	135.41	0.2320	106.13	0.2440	111.61
Isoleucine	0.4685	100	0.5718	122.05	0.5691	121.47	0.6093	130.05	0.5828	124.40	0.6054	129.22	0.5877	125.44
Leucine	0.8378	100	1.0678	127.45	1.0466	124.93	1.1181	133.46	1.0461	124.86	1.1267	134.48	1.0811	129.04
Tyrosine	0.4651	100	0.5742	123.46	0.5721	123.01	0.5976	128.49	0.5555	119.44	0.5961	128.17	0.5791	124.51
Phenylalanine	0.6677	100	0.8379	125.49	0.8904	133.35	0.8151	122.08	0.7766	116.31	0.8126	121.70	0.8265	123.78
Lysine	0.3172	100	0.4021	126.76	0.3747	118.13	0.4048	127.62	0.3925	123.74	0.4070	128.31	0.3962	124.91
Histidine	0.2593	100	0.3470	133.82	0.3454	133.20	0.3570	137.68	0.3501	135.02	0.3642	140.46	0.3527	136.02
Arginine	0.6184	100	0.7326	118.47	0.7116	115.07	0.7926	128.17	0.8000	129.37	0.7546	122.02	0.7583	122.62
Proline	1.3049	100	1.6616	127.34	1.5397	117.99	1.6549	126.82	1.6003	122.64	1.7481	133.96	1.6409	125.75
Cystine	0.2865	100	0.3537	123.46	0.3419	119.34	0.3543	123.66	0.3777	131.83	0.3858	134.66	0.3627	126.60
Total	12.5767		15.7692		15.1695		16.1671		15.5593		16.5039		15.8338	

Of all the various amino acids, it was the histidine and the glycine that increased the most, the average value of each line corresponding to 136.02 and 130.57 percent that of the control. The content of the seven necessary amino acids averaged 122.95 in comparison with the control (which was assigned a value of 100). The valine content averaged 125.57 (ranging from 120.70 to 130.65); the leucine content averaged 129.04 (ranging from 124.86-134.48); the isoleucine content averaged 125.44 (ranging from 121.47 to 130.05); the threonine content averaged 120.32 (ranging from 112.26 to 125.29); the methionine content averaged 111.61 (ranging from 103.80 to 135.41); the phenylalanine content averaged 123.78 (ranging from 116.31 to 133.35); and the lysine content averaged 124.91 (ranging from 118.13 to 128.31).

In summary, there was no great difference in the content of the various kinds of amino acids from one generation to another among the allocytoplasm lines used in this experiment. In the B_7 generation, the content was just a little higher. In the B_7 generation, the amino acid content of 11 of the 17 amino acids was higher than in all other generations. The total content of all amino acids was also higher than in all the other generations, amounting to 131.23 percent that of the nucleus parent. (See Figure 1).

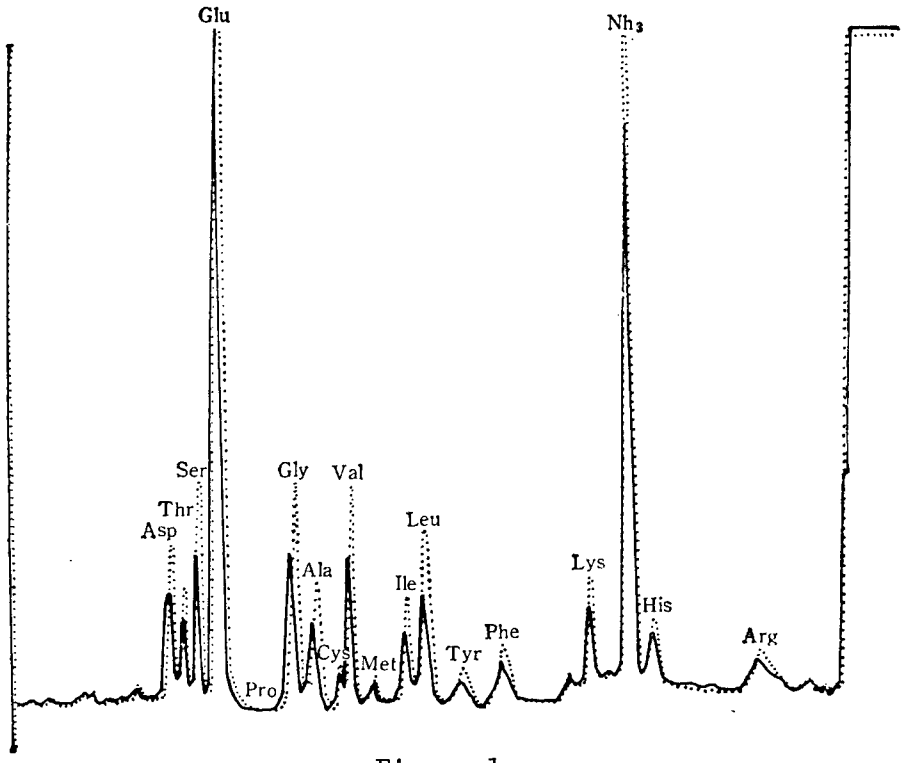

Figure 1
Automatic Recording Results of Amino Acid Column Chromatography
Kenya; ...(squarrosa)-Kenya B_7

Results from the Chinese Academy of Agricultural Science's Comprehensive Analysis Laboratory for 1985 also showed that the lysine content of wheat seeds in all generations of the allocytoplasm line also exceeded that of the nucleus parent variety to a certain extent. The lysine content of the (squarrosa)-Kenya B_6 was 121.95 percent that of the Kenya control.

3. Analysis and Control

Genetic research on the protein and lysine content of wheat grain is an important topic for improvement of wheat quality. In earlier reports, an overwhelming majority of researchers have concentrated on the nucleus genome (the chromosome genome) to the neglect of the genetic effect of the cytoplasm and the nucleo-cytoplasm interaction on quality.

The renowned Japanese scholar, Kihara is a representative of these people who repeatedly engaged in genetic research on the protein and lysine content of wheat allocytoplasm. They discovered that certain cytoplasms, including the cytoplasm of Aegilops squarrosa, could increase wheat grain protein content if they had the proper combination of nucleus and cytoplasm.[1-4] However, most of them reported only the results of single experiments. In the present experiment, we reported the results of repeated experiments conducted over the 3 year period from 1984 through 1986, further demonstrating that Aegilops squarrosa cytoplasm is able to increase the protein content of the grains of common wheat. Furthermore, this genetic effect remained fairly consistent for different years and under different breeding conditions.

In this experiment, we made plantings under similar conditions in the same year in comparative assays of the protein in five generations, B_3-B_7 of seeds of (squarrosa)-Kenya. This made it possible to compare whether there were any differences from one generation to another; and it also enabled maintenance of five allocytoplasm line duplications.

We have already reported that nucleus replacement had been completed in the cytoplasm line of (squarrosa)-Kenya beginning with the B_3 generation. Plant fertility was restored to normal, and morphological and physiological traits, as well as the lipase isoenzyme zymogram were like that of the nuclear Kenya parent.[5] However, results of the present experiment showed differences in the protein content of different generations of seeds, and that there was a trend toward increase in protein content as the number of generations increased. Determination of the mechanism causing this awaits further exploration.

Results of this experiment have shown that by comparison with the nucleus parent variety, the allocytoplasm line (squarrosa)-Kenya B_6 and B_7 generations had a much higher protein content. The 3 year average increase for the B_6 generation was 2.88 percent, and the 3year average increase for the B_7 generation was 2.85 percent. The average content for the two lines was 18.27 and 18.24 percent. Not only does such a result warrant further study, but it holds practical value in and of itself. Our work has also

demonstrated no marked difference in grain yields (small plot yields) for the (squarrosa)-Kenya B_6 and B_7 generations in comparison with the Kenya variety containing the original cytoplasm.[6]

Results of analysis of the amino acid make up of the (squarrosa)-Kenya allocytoplasm line grain showed an identical overall trend as results of the analysis of the aforementioned protein content. When lysine content is expressed in terms of a percentage of dry material, the seed protein content and lysine content show a positive correlation, Kihara also obtained an allocytoplasm line of wheat in which the protein and lysine contents were high. It not only was greater than that of the nuclear parent variety, but also higher than that of top quality control varieties.[3]

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9432/9604

Experiments in Synthesizing Natural Blood Pressure Regulating Chemical

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[Article by Qin Ning [4440 1337] and Li Yuyang [2621 5148 7122], Genetics Institute, Fudan University, Shanghai: "Synthesis and Secretion in Yeast of Alpha Human Atrial Natriuretic Polypeptide Under Control of Alpha Factor Gene Promotor-Signal Sequences"]

[Text] a-h ANP is a polypeptide hormone that is secreted by cardiac muscle cells and cardiac atrium tissue in mammals. It plays a powerful nutritive and diuretic role, and a powerful role in the expansion of blood vessels and the lowering of blood pressure. It holds very great hope for becoming one of the optimum medicines for treatment of illnesses such as high blood pressure. Since a-h ANP possesses such extremely attractive prospects for use, we used a chemically synthesized alpha human atrial natriuretic polypeptide (a-hANP) gene that had produced a secretory expression under control of saccharomycete sucrose (SUC 2) promotor-signal sequences. Recently we further used the promotor-signal sequences of a yeast alpha factor gene to produce a fairly good secretory expression by an alpha-hANP gene in saccharomycetes.

The carrier we used for expression was YFD18, which is a yeast-E. coli shuttle plasmid that has yeast LEU2 nutrient marked genes, a 2 alpha mu plasmic replication zone segment, and a yeast alpha factor gene promotor-signal sequence。 Lower down it has a Hind III enzyme single cutting point. After the plasmid was cut with Hind III enzyme, it was processed in an alkaline phospholipase。 Then it was connected to a Hind III segment containing the chemically synthesized alpha-hANP gene, and the SUC2 gene in the transcribed termination zone. After E. coli MC1061 transfer, the colony in situ hybridizing method was used to select recons containing alpha-hANP inserted segments. Then an assay was done using Hind III enzyme cutting and polyacrylamide gel electrophoresis to verify that the hybrid positive colony contained approximately 500 bp of Hind III segment inserts containing the alpha hANP gene. Since the Hind III enzyme single cutting point had been used with the recon, there were two possibilities for the direction of insertion of the alpha-hANP segment. Therefore, we randomly selected four recons for transfer to the saccharomycete Y33 (alpha, his, ura, leu, ade, suc), and we used the radioimmunoassay method to assay

the alpha-hANP's expression. Results showed two recons able to express alpha-hANP, which were named YFD42. We also assayed the amount of alpha-hANP within cells, in spaces around cells, and in the culturing solution, verifying the alpha-hANP content of the first two kinds of cell components to be less than 1 mu gram per L^3, and 500 mu grams per L^3 in the culturing solution. This result showed that the yeast alpha-factor promotor-signaling sequence was effective in directing the alpha-hANP synthesis and secretion. In particular, when secretion reached a fairly high level, this greatly simplified the isolation and purification of genetically engineered products. Currently, we are in the process of isolating and purifying expression products, and further assaying the alpha-hANP synthesized by the system.

9432/9604

Successful Preliminary Cloning, Expression of Asparaginase Gene

40081082c Beijing KEXUE TONGBAO in Chinese Vol 33 No 5, Mar 88 p 399

[Article by Guan Yingqian [7070 4481 6197], Chen Jianmin [7115 0494 3046], Peng Huilin [1756 1920 2651], He Yunsheng [0149 0061 3932], and Jiao Ruishen [3542 3843 6500], Shanghai Plant Physiology Institute, Chinese Academy of Sciences: "Preliminary Cloning and Expression of Leukemia Drug Asparaginase Gene"]

[Text] Microbial asparaginase is effective in the treatment of different kinds of cancers such as leukemia, lymphosarcoma, and carcinosarcoma, and it produces excellent results in the treatment of various kinds of acute leukemia. The asparaginase that has been used in treatment since the 1960's has come from E. coli, and though treatment results have been good, there have also been certain side effects. In recent years, microbes capable of producing asparaginase have been found including Erwinia carotovora, Serretia marcescens, and Vibrio succinogenes. Of these, glutimaminase activity is extremely low in Vibrio asparaginase preparations; hence their specificity is higher, and side effects few. However, asparaginase output from Vibrio anerobes is very low. It was for this reason that we applied genetic engineering techniques to increase the asparaginase output of Vibrio anerobes in order to develop a new source of enzyme preparations for the treatment of leukemia of high effectiveness and low toxicity in China. This article reports the preliminary cloning work that has been done on Vibrio asparaginase genes.

For a carrier, we used expressive bacteriophage λgt 11, and ^{125}I marked radioimmunoantibodies as a probe, cloning segments from Vibrio succinogenes chromosome DNA that had been cut by enzymes from EcoRI to target segments carrying the asparaginase gene, obtaining expression in a host microbe, E. coli-Y1090. From 56,128 recombinant bacteriophage plaques, we obtained nine recombinant bacteriophage plaques containing target gene segments, and we used EcoRI on five strains of them to carry out enzymolysis and electrophoresis analysis. The size of the resultant inserted DNA segments were identical at 5.8 kb showing that we had achieved a true cloned strained. After the recombinant DNA infected another host E. coli-Y-1089, the Nessler's reagent chemical colorimetric method was used to assay the

asparaginase activity of in its cell-free extract. When recombinant DNA (λgtll-AS8) was used as a probe in Southern DNA hybridizing, a hybrid band located at 5.8 kb appeared in the EcoRI enzyme cut segment of the Vibrio succinogenes chromosome DNA. This proved that the target segment carrying the asparaginase gene that we had cloned had come from the Vibrio succinogenes, and that expression had been achieved.

Results of the preliminary cloning, molecular hybridization, chemical method assay, and electrophoresis show the 5.8 kb DNA segment we obtained contains the Vibrio asparaginase gene.

9432/9604

Cloning of Yeast Gene Into E. Coli

40081085a Beijing ZHONGGUO KEXUE [SCIENTIA SINICA, SERIES B] in Chinese No 5, May 88 pp 490-498

[Article by Jiang Weidong [1203 0251 2639] and Kuang Daren [0562 65671 0086], Shanghai Cell Biology Institute, Chinese Academy of Sciences: "Cloning, Expression, and Assay of Saccharomyces Cerevisiae PRO3 Gene"; a National Natural Sciences Fund Assistance Project]

[Text] Abstract: Study of pathways for the synthesis and metabolism of proline in yeast and their enzyme gene has only begun in recent years. We cloned a yeast PRO2 gene into bacteria and went on to clone the PRO3 gene. Such cloning is not only able to complement the yeast very well, but it also finds highly effective expression in the bacteria, and very high enzyme activity is detectable. This shows that this gene is able to use its transcription and translations systems in both eukaryocyte yeasts and prokaryocyte bacteria. After the PRO3 gene has been cloned to a multiple-copy plasmid, activity of the gene products is no higher than on single-copy chromosomes in either yeasts or bacteria, showing this gene's expression to play a regulatory role.

The code P5C reductase (L-Proline: NAD(p^+) 5-oxidoreductase; E.C. 1.5.1.2.) gene ProC in E. coli has been gene mapped[1], and cloned[2], and its sequencing has also been completely analyzed.[3] Deutch et al.[3,4] also studied thoroughly the regulation in E. coli of the way in which proline is biologically synthesized. When the P5C reductase gene is cloned to a multiple-copy plasmid, its activity exhibits no dose effect; however, when switched on by the bacteriophage promoter, P_L, induced by a temperature of 42°C, it will overproduce P5C reductase.

It was not until 1979, when Brandriss[5] of the Massachusetts Academy of Science and Engineering isolated a proline nutrient deficient mutuant strain of Saccharomyces cerevisiae that research began on the Saccharamomyces cerevisiae gene. Yeast and E. coli are very similar in that the mutant strains of the E. coli may be divided into three complementary groups corresponding to the three enzyme gene mutants in the biological synthesis of coded proline, namely PRO 1, PRO 2, and PRO 3, PRO 3 being the code P5C reductase gene, corresponding to the pro C in E. coli. An extraordinary characteristic is that yeast proline-deficient strains are unable to grow in

rich yeast paste, peptone, or glucose media, and this is the reason that study of the proline gene is difficult. It was later found that this inability to grow in these media resulted from the existence in them of ammonium ions, which stopped the action of the yeast transport system so that the yeasts, which were themselves lacking the ability to synthesize proline, could not use the proline in extracellular media, and were thus unable to grow.[6]

Recently our laboratory cloned and assayed the PRO2 gene in yeast[7], finding the PRO2 gene able to effectively complement the E. coli proA mutant strain; however, it could promote the E. coli's ability to tolerate and diffuse salt. This article reports the use of shuttle plasmid YRP7[8] to construct a saccharamoycetes gene pool and to clone a PRO3 gene from it. This PRO3 gene was also able to effectively express and complement in E. coli. We have made a preliminary study of this gene's structure and regulation of enzyme activity.

I. Materials and Methods

1. Bacteria and Plasmids

E. coli: C600: hsdR, hsdM, thr, leu, thi, lac, F^-; HB101; recA13, supE44($su2^+$), lacZ4, leuB6, proA2, thi, Sm^R, hsdR, hsdM, F^-, gal; JM83:ara, Δ(lac pro), thi strA, lsdR, hsdM, LacZΔM15, F^-, DH_1:F^-, recA1, endA1, gyrA96, thi-1, hsdR17, supE44; X342 (CGSC4515; proC29, metB1, relA1, spoT1, λ^-, (Bachmann donated); LCK8 (CG5C6515:F^-, λ^-, supE44, lac-3 or lacY1, galK2, galT22, metB1, hsdR2, zjj-202::Tn10 (Bachmann donated); X28: proC29, metB1, relA1, spoT1, λ^-, hsdR2, zjj-202::Tn10, (obtained from the modification of X342; see this article).

Saccharomyces cerevisiae: Eal-103:α, ura3, trp1, leu2; MB299-7A:α, pro3; 1y3, (Brandriss donated[5]); 7209-1A:α, wild type (donated by Cai Jinke [5591 6855 4430].

Plasmids: YRP7:Ap^r, Tc^r, TRP1, ARS1, 5.8kb[8]; YCP19; Ap^r, TRP1, ARS1, CEN4, URA3, 10.6kb[9].

P1 bacteriophage (fulminating).

2. Culture Medium and Enzymes

Large cell baccilus medium: Refer to Miller[10] method.

Culture used with yeast: Refer to Sherman[11] method. For the medium used with bacterium MB299-7A, refer to Brandriss[5] method.

Restriction endoclase: EcoR1, HindIII, BamHI, SalI, PstI T_4 DNA ligase (made ourselves); Sau3A(BRL); DNA polymerase I and cochlease [5815 3662 5326] (Beijing Biophysics Institute); DNasel (Sigma). For enzyme snipping and linking conditions, refer to Methods in Enzymology, volume 100.[12]

3. Chemical Reagents

Radiation isotopes: α-^{32}P-dATP, α-^{32}P-dCTP (3000mCi/mmol, 1mCi/ml 50 percent alcohol solution or 10mCi/ml STE solution, Amersham); low dissolving point agarose (BioRad); reduction type coenzyme II (NADPH) (Sigma); strongly acidic positive ion exchange resin (Dowex, 2 percent cross linking, Shanghai Organic Chemicals Institute pilot plant); P5C precursor (CalBiochem); cyclosermase (Sigma).

4. DNA Preparation

Plasmid DNA extracted using the Birnboim and Doly[13] and the Zasloff[14] method. Rapid micro DNA preparation done using the Maniatis[15] method with slight modifications. Total DNA extraction from yeast was done using the method in reference [16].

5. Transduction and Transformation of E. coli and Yeast Transformation

Slightly simplified Morrison[17] method referred to in the transformation of E. coli, processing of the $CaCl_2$ and the $MgCl_2$ being done in a single step ice bath for 20 minutes, the concentration of the $MgCl_2$ dropping to 0.02mol/L. Transduction of the E. coli was done according to the Miller[10] method. Two methods were used in the transformation of the yeast, namely the Hinnen[18] protoplast method and the Ito[19] lithium acetate method.

6. E. coli Transformation for Screening Ap^rTc^s Using the Cycloserine Method

Refer to the Bolivar and Backman[20] method. Time of cycloserine processing of cells differed with different bacteria.

7. Mitosis Stability Testing of Plasmids in the Yeast

Testing of the stability of mitosis in the Saccharomyces cerevisiae Ea1-103 plasmids was done in accordance with reference [21]. Since bacterium MB299-7A was unable to grow in the YEPd medium[5], it was grown in a special nonselective medium SD/Pro/Lys, a 2D replacing the growing of a 1d in the YEPd. The single colonies produced were then grown again in a selective medium (SD/Lys) and a nonselective medium (SD/Pro/Lys), and the percentage of plasmids still growing in the yeast after approximately 20 generations was calculated. It was these plasmids that were used as the index of mitosis stability.

8. Nucleic Acid Hybrid Experiment

Performed as described in reference [22] and [23].

9. P5C Preparation

Prepared according to the Calbiochem products instruction booklet and reference [24].

10. Preparation of Crude Protein Extract and Assay of P5C Reductase Enzyme Activity

Protoplasts were obtained by using the method found in reference [16], and preparation of the crude protein extract was synchronized with the E. coli.[25] Then, at 340 mm, a Beckman Model DU-7 ultraviolet spectrometer was used to record the decline in absorption value.

II. Results

1. Use of Shuttle Plasmid YRp7 To Construct a Yeast Gene Pool

Chromosome DNA from a wild yeast, 7209-1A, served as the donor. This was partially enzyme cut at Sau 3A and low dissolving point agarose gel electrophoresis was used to isolate a 4-20kb long DNA segment, which was spliced into the YRp7 at the BamHI location. Then HB101 and C 600 were transformed, and the Ap^r transformant was screened out. Approximately 90 percent of the 5×10^6 Ap^r that was obtained was Tc^r. Therefore, the cycloserine method was used to eliminate most of the autocycled plasmid (Tc^r), and the plasmid was extracted. The recombinant plasmid was separated from the carrier YRp7 using low dissolving point agarose gel electrophoresis. The HB101 was retransformed and the recombinant plasmid enriched to serve as a gene pool. More than 10 individual colonies were selected at random from among the Tc's HB101 transformants for the extraction of plasmid DNA, which was subjected to electrophoresis to see whether it was larger than the YRp7. Figure 1 [not reproduced] shows that the Ap^rTc^s transformants did indeed contain inserted segments of recombinant plasmids of various sizes.

Several Ap^rTc^r individual colonies were also selected from the gene pool for analysis of their plasmids, several of which were also found to be bigger than the YRp7, and of different sizes. This showed that very possibly they too had been inserted. If so, by using the gel electrophoresis method to isolate recombinant plasmids, loss of Tc^r transformant could be avoided. Moreover, either strict cycloserine processing or some other method might produce an incomplete gene pool.

2. Modification of E. coli X 342 ($proC^-$) and Cloning of the PRO3 Gene in the E. coli

The gene pool was used to transform E. coli proC mutant strain X342. Consequently, the transformation rate was exceptionally low (see Table 1), even to the point where it was not very possible to obtain complementary transformants. This may have been because of the X342's $hsdR^+$, which restricted the entry of this kind of heterogenous DNA into the yeast gene pool. Therefore, we figured out a way of modifying it to become an $hsdR^-$ bacterium.

With E. coli LCK8 as a DNA donor, we used P_1 bacteriophages in the total transduction to X342. Since there was a Tn10 transposition at the zjj-202 site near the $hsdR^-$ gene in the LCK8, it had an obvious Tc^r screening label. In the genetic diagram for E. coli LCK8, the genetic diagram distance between hsdR and zjj-202 was approximately 0.4 minutes; therefore, the total transduction frequency between them was estimated at more than 30 percent.

Table 1. Transformation Frequency of Different E. coli Strains Using Different Plasmids

Bacteria strains	Plasmid DNA				
	Control (TE) (Ap^r)	pBR322 (Ap^r)	YRp7 (Ap^r)	YRp7 Pool (Ap^r)	YRp7 Pool (Ap^r Pro^+)
X342	0	10^5	10^3	10^3	0
X28	0	10^6	10^6	10^6	10-100

Plasmid YRp7 was used to transform the Tc^r from the X342. In 5 of 12 Tc^r bacteria the frequency of YRp7 transformation was markedly higher than for the X342 bacteria before modification. The eighth transductant (namely bacteria strain X28) served as the receptor in gene pool transformation, and a higher transformation rate was obtained (see Table 1). Assay of the X28 nutrition deficiency showed it still to be the same as for X342, showing that the X28 was truly modified from the X342, and was not a stray pollutant.

Next, 1-5 μg of gene pool plasmid DNN transformed proC-deficient E. coli X28, and Ap^r, and ProC complementary transformants were screened out. From more than 100 transformants (see Table 1), we selected two individual bacteria colonies from which we extracted their plasmids pCBy203 and pCBy204. We used PstI, EcoRI, and HindIII in preliminary enzyme snipping to show that these two plasmids contained identical inserted segments. We used the pCBy203 to retransform the X28, attaining a transformation rate as high as for the YRp7, and that was also expressed as Ap^r and ProC complements. This showed the E. coli ProC complement as having genuinely been derived from plasmid pCBy203.

3. Limited Site Analysis of the PRO3 Gene Segment, and Preliminary Identification of the Minimum Functioning Zone Complementary to E. coli ProC

We used restriction endoclase PstI, EcoRI, BamHI, and SalI on the pCBy203 plasmid in a detailed enzyme cutting analysis, the results of which are shown in Figure 2. There were two PstI cutting points on Y203 of this ProC gene segment, namely one HindIII and one BamHI cutting point; however there were no EcoRI and no SalI cutting points. A rough calculation of its molecular weight is probably 2.7kb. Subsequently, a preliminary shortening of the functioning zone was done.

Double enzyme cutting was performed on the pCBy203 using PstI and EcoRI or BamHI and HindIII. Then, the shortened restriction enzyme cut segment obtained was cloned into pWR13. This sub clone transformed the X28, with the result that just ProC complementary X28 transformant (its plasmid being pCBy213) was derived from the PstI/EcoRI enzyme cut and joined specimen. Further restriction enzyme cutting analysis revealed that the yeast PRO3 gene functioning area that is complementary to the E. coli ProC mutant strain is in the middle of the Y203 gene on the 2.3kb segment between the PstI cutting point and the Sau 3A clone site (Figure 3).

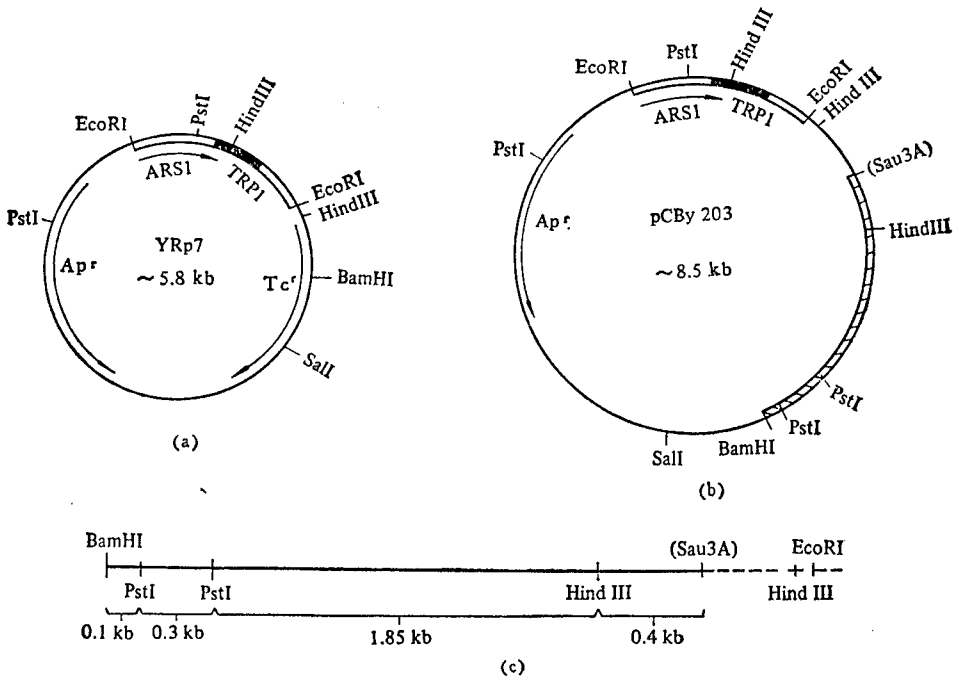

Figure 2. pCBy203 constructed by cloning the 2.7kb PRO3 gene segment to the YRp7 BamHI position. Figure is the pCBy203 restriction enzyme cutting atlas.

((a) YRp7--carrier used in cloning, (b) pCBy203--this plasmid contained the 2.7kb PRO3 gene segment ( ) that was cloned to YPp7, (c) 2.7kb gene segment)

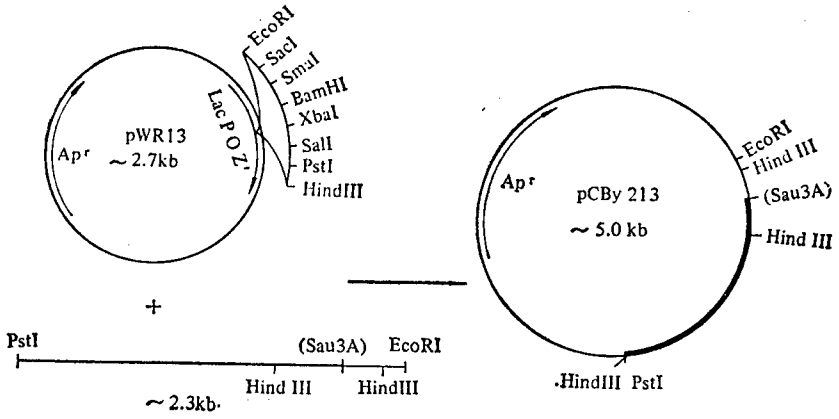

Figure 3. pCBy213 was constructed by cloning the PRO3 gene functioning zone to the sequence-finding plasmid pWR13. Figure is a schematic drawing showing construction. (—— is the PRO3 gene segment)

4. Transformation of the PRO3 Gene in the Yeast MB299-7A (PRO3) and Assay of the Transformant

Our initial goal in setting up the yeast shuttle plasmid gene pool was the direct transformation of yeast PRO3 mutant strains. We used both the Hinnen protoplast method[18] and Ito's LiAc method[19] in the simultaneous transformation of MB299-7A, with the result that the same number of PRO^+ bacteria colonies were obtained from the control to which no plasmid had been added. However, with no addition of DNA to the control bacteria Eal-103, no TRP^+ colonies appeared (Table 2). This shows that the yeast MB299-7A may possibly have a fairly high frequency of reverse mutation, or that there are other reasons that change the nutrient deficiency. Therefore, use of the gene pool in the direct screening of the PRO3 gene from the yeast MB299-7A is very difficult. Consequently, the aforementioned method was reverted to in order to screen from the bacteria a yeast gene segment that was complementary to the ProC.

Table 2. Frequency of Yeast Transformation

Bacteria strain	Genetic marker	Specimen used in the transformation		
		Control (TE)	YRp7 pool	pCBy203
Eal-103	TRP^+	0	10^3	10^3
MB299-7A	PRO^+	3-6	3-6	10^2

After obtaining the ProC complementary yeast plasmid pCBy203, we again transformed the yeast PRO3-deficient strain MB299-7A. Use of the simple LiAc method produced more than 100 transformants (Table 2), which was markedly greater than the frequency of appearance of $PRO3^+$ from the MB299-7A that was directly transformed by the control specimen and the gene pool. This showed that the pCBy203 plasmids did indeed contain functioning segments that were complementary to the yeast PRO3 mutant strain. As to why the MB299-7A transformation rate was lower than that of the Eal-103, possibly it was because the MB299-7A was unable to grow in a rich culturing medium. Growth in a medium to which proline and lysine had been added was not as good as growth of Eal-103 in a rich culturing medium. Creation of different physiological conditions caused differences in cell receptivity. Another possibility is that the complementary function of the TRP1 may be stronger than that of the PRO3. This situation did not occur when MB299-7A was used in a rich medium, and the $PRO3^+$ transformant could be directly screened in the rich medium, or a rich medium could serve as a selective pressure for the culturing of $PRO3^+$ transformants.

By way of further verifying that the PRO3 complement was induced by a free pCBy203 plasmid, we ran a detailed assay of the $PRO3^+$:

1. We extracted all of the DNA from the $PRO3^+$ transformant of the MB299-7A, and then transformed the E. coli X28, rescreened the Ap^rProC^+, and extracted the plasmids. Enzyme cutting assay found it was identical with the original pCBy203, showing that the pCBy203 existed in the yeast cells in free form. After extraction, it was transformed into complementary ProC in E. coli.

2. Mitosis Consistency Test of the Plasmid pCBy203 in the Yeast Cells. Without a selective pressure, the YRp7 plasmid in the yeast might very easily be lost. If the genetic marker gene conformed to the chromosome, or if the marker gene on the chromosome produced reverse mutation, this gene's phenotype would be fairly consistent in the mitosis process. Thus, observation of the stability of the mitosis process could be used to separate the transformation caused by the free plasmids from other reasons.

We selected two individual bacteria colonies (203-1 and 203-2) from the MB299-7A PRO3$^+$. After growing 2d in SD/Pro/Lys nonselective medium, we divided the individual colonies in SD/Pro/Lys, putting approximately 100 individual colonies in separate culturing media of SD/Lys and SD/Pro/Lys. By calculating the ratio of the number of colonies that grew in the SD/Lys and the SD/Pro/Lys, we learned the percentage of bacteria colonies that retained plasmids (see Table 3). In addition, conventional methods were used to test the stability of plasmid pCBy203 in Eal-103.[21] After growing in nonselective medium YEPD for 1 day, the individual colonies were divided and put into YEPD and SD/Leu/Ura media, and the number of retained TRP1$^+$ bacterial colonies calculated. Since MB299-7A cannot grow by itself in a rich medium of YEPD, while PRO$^+$ transformants can, we were able to use only the SD/Pro/Lys as a nonselective medium. However, since basic medium nutrient conditions varied, a fairly long culturing time was required before a comparison could be made with the growth of Eal-103 in the YEPD, a growing cycle of approximately 20 generations being reached.

Table 3. Mitosis Stability of Various Plasmids in Different Yeasts

Plasmid/yeast strain	Genetic marker	Non-selective medium	Selective medium	Percent of residual plasmids after 20 generations
pCBy203MB299-7A	PRO3	SD/Pro/Lys	SD/Lys or YEPD	1-2
pCBy203MB299-7A	PRO3	SD/Pro/Lys	SD/Lys or YEPD	1-2
pCBy203/Ea-1-103	TRP1	YEPD	SD/Leu/Ura	1-2
RP7/Eal-103	TRP1	YEPD	SD/Leu/Ura	1-2
Ycp19/Eal-103	TRP1	YEPD	SD/Leu/Ura	98

Comparison of the expression of the YRp7 and the pCBy203 in the Eal-103 with the expression of the pCBy203 in the MB299-7A (Table 3) showed them to be similar. Comparison of the high consistency in the Eal-103 of the Ycp19 plasmid that contained CEN4 showed no equivalent type structure containing CEN in the pCBy203, much less pCBy203 plasmid conformity, or the creation of a PRO3 complement by PRO3 reverse mutation. This shows that the complementary action of the PRO3 is brought about by the existence of the pCBy203 plasmic.

3. Southern Hybridizing Assay. After double enzyme cutting of the pCBy213 by PstI and HindIII, agarose gel with a low dissolving point was used to isolate a 1.85kb segment (see Figure 3) as a probe for Southern spot hydridizing and print hybridizing.

(1) Southern Dot Hybridizing. Results are shown in Figure 4 [not reproduced]. This figure shows strong hybridizing of the cloned Y203 segment only with the total yeast DNA, and only weak hybridizing with cell DNA, which may very possibly be nonspecific hybridizing. However, because it was protected at the time of prehybridizing, the calf thymus gland DNA did not show even any nonspecific hybridizing, showing that the PRO3 gene segment that we cloned came from the yeast.

(2) Southern Print Hybridizing. Total DNA was extracted from the yeast transformant. Following electrophoresis, Southern print hybridizing was begun (see Figure 5 [not reproduced]). Electrophoresis showed the existence in the MB299-7A of not only a 2μ plasmid, but a very small plasmid as well, and the visible bands of these two were unable to hybridize with the PRO3 gene following electrophoresis. During electrophoresis, no plasmids were visible in the Eal-103 and its pCBy203 transferrant; however, a pronounced plasmid band was visible in the pCBy203 transferrant of the Eal-103 as a result of hybridizing that was identical to the pCBy203 plasmids in the MB299-7A and the X28.

5. PRO3 Gene Products--Determination of P5C Reductase Activity in E. coli and Yeast

P5C reductase is the third step reaction enzyme in the biosynthetic pathway of the catalysis of proline. Gamma-glutamic acid semi-aldehyde

$$\xrightarrow[\text{PSC reductase}]{\text{NAD(P)H NAD(P)}^{+}} \text{proline,}$$

is able to test activity directly in a crude extract taken from E. coli and yeast. Figure 6(a) shows the level of enzyme activity in E. coli ProC-deficient strain X28 after PRO3 gene transfer. Here one can see clearly that activity may be tested only after the use of yeast PRO3 gene transfer. However, in wild E. coli DH_1, after transfer of multiple-copy plasmid pCBy203 containing a PRO3 gene, the P5C reductase activity did not increase (Figure 6(b)). In yeast, the expression was similar to that of this gene in E. coli. In PRO3 mutant strain MB299-7A, the PRO3 gene on the multiple-copy plasmid pCBy203 was able to express effectively (see Figure 6(c)); however, when transferred into the $PRO3^{+}$ yeast Eal-103, activity did not increase (Figure 6(c)). This shows that the PRO3 can express very well in both E. coli and yeast; however, the number of its copies had no effect on the activity of its gene products.

III. Discussion

Glutamic acid biosynthesis of proline requires three enzyme parameters and reactions, namely, glutamic acid kinase (enzyme I), gamma glutamic acid semi-aldehyde (enzyme II), and PSC reductase (enzyme III). Enzyme I and enzyme II are coded by an operon in a cell, which is a rate-limiting step in this synthesis pathway[4], the activity of which is regulated and

controlled by feedback from the end product proline. The activity of these two enzymes in the cells is also very low, which helps the regulation and control. Enzyme III is more active in the cells.[3] It is not controlled by the end product. Expression and regulation of the three enzyme genes in the yeast that biosynthesize proline is similar to that of E. coli. The yeast PRO2 gene that we cloned in our laboratory made the E. coli more salt tolerant[7] in the same way as the ProBA of salmonella.[26] The yeast PRO3 gene reported in this article is similar to the bacteria's ProC[3] in that they had no marked genetic dose effect in either E. coli or in yeast. In E. coli, the ProC gene can increase 200-fold the activity of P5C reductase at 42°C when switched on by a bacteriophage promoter, PL, showing that the key to controlling this enzyme's activity rests with the promoter.

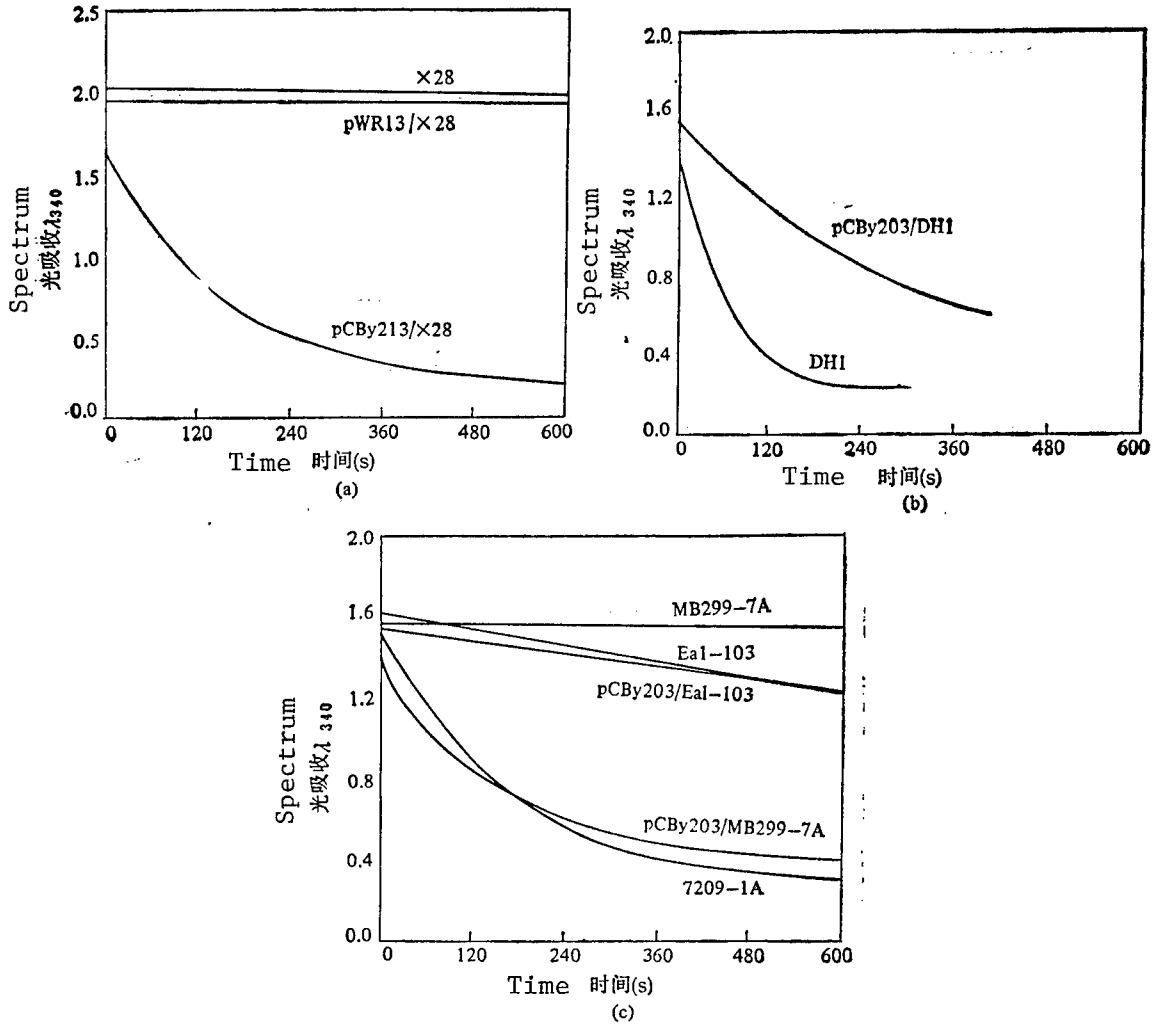

Figure 6. Comparison of P5C reductase activity of each yeast.

Since yeasts are eukaryotes, yeast transcription and translation systems are different than in the prokaryotic E. coli. We cloned a PRO3 gene from yeast to E. coli, and we were able to detect activity of the PRO3 gene products in the E. coli. This may explain these problems as follows: 1) This gene's promoters are also able to be recognized by the E. coli's transcription system, or a structure remains in the promoter position that is similar to the E. coli promoter that causes the PRO3 gene to transcribe and generate functioning mRNA; 2) in the E. coli there is no eukaryote mRNA cap and poly(A) caudal structure additional system, but the mRNA is still able to translate into functional P5C reductase.

This article has laid a foundation for thorough study of the PRO3 gene. The shortening of the functioning area, and the cloning to the pWR13 plasmid helps in the task of analyzing PRO3 gene sequencing.

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9432/6091

Study of Recently Discovered Sugar Metabolizing Enzyme, PFK-2

40081085b Beijing ZHONGGUO KEXUE [SCIENTIA SINICA, SERIES B] in Chinese No 5, May 88 pp 499-504

[Article by Li Lin [2621 2651] and Xu Genjun [6079 2704 0193], Shanghai Biochemistry Institute, Chinese Academy of Sciences: "Purification and Study of the Dynamics of Mouse Liver Fructose 6-Phosphate, 2-Kinase"; a project assisted by the National Natural Sciences Fund]

[Text] Abstract*: A series of processes was used to purify mouse liver fructose 6-phosphate and 2-kinase including ultracentrifuging, polyethylene glycol fractional precipitation, and DEAE-Sephadex, Blue-Sepharose, and phosphate cellulose column chromatography. Gel filtering and the SDS polyacrylimide electrophoresis method determined the enzyme to be made up of two similar subunit proteins having a molecular weight of 110,000. Mg^{2+} was necessary for its activity, and the actuation method was positively synergistic. The method of combining the enzyme with the substrate exhibited direct synergism with the fructose 6-phosphate, but no synergism with ATP. Its Km value increased as the concentration of fructose 6-phosphate decreased, showing the role of the enzyme to be that of a sequencing mechanism. The activity center is related to a requisite amino acid combined with ATP. Following combination, its lateral chain pKa shifted from 9.5 to 9.8.

PFK-2 is a new enzyme involved in the metabolism of sugar that was reported virtually simultaneously in 1981 by Pilkis et al.,[1] Hers et al.,[2] and Uyeda.[3] Its importance derives not from its location in a key position that is a normal sugar metabolism route, but that it catalyzes Fru6P and ATP reactions to form the products Fru2, and $6P_2$ that have an important regulatory role in the metabolism of sugar. Fru2, and $6P_2$ activate the PFK-1, while simultaneously exerting a power inhibiting influence on fructose 1,6-diphosphoesterase. It is interesting that PFK-2 can be phosphorylated by protein kinase and express fructose 2,6-diphosphoesterase activity.

*The following abbreviations are used in this article: PFK-2 for fructose 6-phosphate, 2-kinase; PFK-1 for fructose 6-phosphate, 1-kinase; Fru6P for fructose 6-phosphate; Fru2,$6P_2$ for fructose 2,6-diphosphate; PEG for polyethylene glycol; PMSF for p-methyl sulfonyl fluoride; and PFP for pyrophosphoric acid: fructose 6-phosphate, 1-phosphate transferase.

After the protein phosphoesterase dephosphorylates, it again expresses as kinase.[4] It is a special enzyme that has characteristics of both kinase and an esterase catalyst.[5] Study of the correlation between the structure and function of PFK-2 will help clarify the functioning mechanisms of both kinase and esterase and the relationship between them.

I. Materials and Methods

Male, ICR mice weighing 18 grams each provided by the Shanghai Experimental Animal Center. Fru6P, a product of the Bohringer Company; Fru2, and $6P_2$, a product of the Sigma Company; ATP, a product of the Shanghai Dongfeng Biochemical Reagents Plant; and PEG 6000, a Serva Company product.

Blue sepharose 4B, using epichlorohydrin crosslinked sepharose 4B and blue active dye provided by the Shanghai No 8 Dye Plant.[6]

Purification of Mouse Liver PFK-2. The livers (weighing approximately 135 grams) were taken from 150 mice within 1/2 to 1 hour after they had eaten, and were chilled in three times the volume (V/W) of buffering solution A (20 mmol/L Tris-HCl, pH 7.8, 50 mmol/L KCl, 0.5 mmol/L EDTA, and 2 mmol/L mercaptoethanol). Then they were homogenated in a pounding machine (20s x 2) after which PMSF propanol solution was added to a concentration of 0.5 mmol/L (all the following steps being carried out at below 4°C). After centrifuging (30,000 g x 30 min), the supernate was recentrifuged by ultracentrifuge (108,000 g x 60 min), the sediment discarded, and then solid PEG 6000 added to the supernate to 6 percent (W/V) concentration. The supernate was let stand for 15 minutes after which it was centrifuged (30,000 g x 10 min) and PEG 6000 was added at 12 percent (W/V). The precipitate was collected, and after it was dissolved in 80 ml of buffering solution A, a DEAE-Sephadex A-50 column (2.0 x 14 cm) was used for adsorption. After washing in 150 ml of buffering solution containing 0.1 mmol/L KCl, between 0.1 and 0.5 mmol/L KCl (each 125 ml) gradient elution was done.

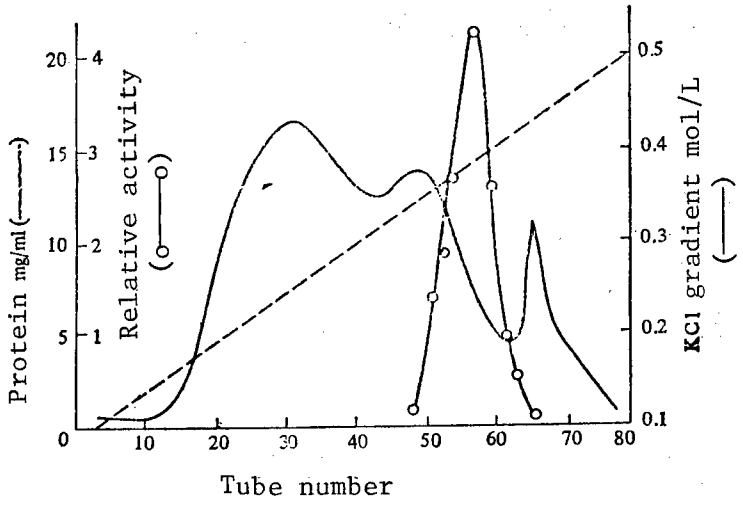

Figure 1. DEAE-Sephadex A-50 Column Chromatography Isolation of Mouse Liver PFK-2

The active portion of the PFK-2 was added to the buffering solution B (20 mmol/L Tris-HCl, pH 7.3, 50 mmol/L KCl, 0.5 mmol/L EDTA, and 2 mmol/L mercaptoethanol) for dialysis, then isolation was done using a Blue-Sepharose 4B column (2.0 x 9 cm). After the column was washed in 100 ml of buffering solution B, 1 mol/L KCl eluted the PFK-2. The specimen was collected and added to buffering solution C (20 mmol/L Tris-HCl, pH 7.5, 10 mmol/L KCl, 0.5 mmol/L EDTA, and 2 mmol/L mercaptoethanol) for dialysis equilibration; then it was placed in a -20°C refrigerator.

PFK-2 Activity Assay. For the method used to keep the sediment warm, refer to the method reported by Pilkis et al.,[7] including Fru6P 2 mmol/L, ATP 5 mmol/L, and a reaction temperature of 30°C. Fru2,$6P_2$ quantitative data, and the extent of its role in activating PFP was as reported by Hers et al.[8] The known concentration for Fru2, $6P_2$ was the standard. Under these conditions, each unit of enzyme is to catalyze the production of 1 u mol Fru2, $6P_2$ of enzyme per minute.

Potato PFP Preparation. The method of Hers et al.[8] was used.

Protein Concentration Assay. The Coomassie brilliant blue method[9] was used, taking ox blood serum protein as the standard.

II. Results

1. Purification of Mouse Liver PFK-2

See Table 1 for matters pertaining to the purification process. The enzyme was purified 2,600-fold in the entire process, the yield being 13.4 percent.

Table 1. Mouse Liver PFK-2 Purification

Steps	Total protein (mg)	Total vitality (mU)	Proportional vitality (mU/mg)	Purification multiple	Yield rate (%)
Ultracentrifuged supernate	12,509	122	0.01	1	100
12 percent PEG sediment	6,728	99	0.015	1.5	81
DEAE-Sephadex	290	65	0.22	22	53
Blue-Sepharose	139	61	0.44	44	50
Phosphate cellulose	0.64	16.4	26	2,600	13.4

The purified PFK-2's molecular weight assayed at 110,000 following Sephadex G-150 filtering. SDA polyacrylamide electrophoresis showed an attenuated zone. Molecular weight was 55,000. This showed that the mouse liver PFK-2 was an oligomerase formed from two similar subunits.

2. Effect on PFK-2 of Bivalent Metal Ions

Figure 2 shows the correlation between Mg^{2+} and PFK-2 activity. ATP was 0.2 mmol/L, and Fru6P was 2 mmol/L in the enzyme reaction sediment. Mg^{2+} is necessary for PFK-2 catalyst activity, and neither Mn^{2+} nor Zn^{2+} can take the place of the Mg^{2+}. The activation of the Mg^{2+} is synergistic, and the Hill coefficient is 3.6.

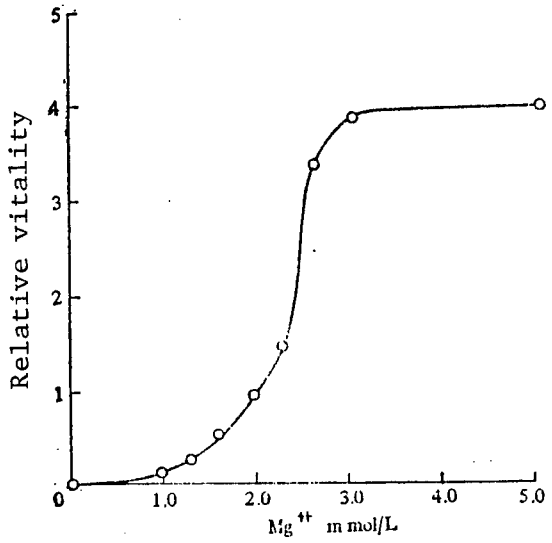

Figure 2. Activation of PFK-2 by Mg^{2+}

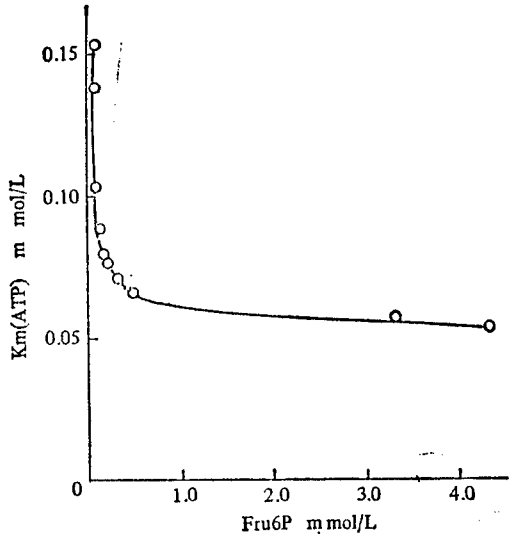

Figure 3. Km(ATP) Effect on PFK-2

3. Sediment Combining Method

The PFK-2-Fru6P saturation curve also shows a synergistic effect. At a constant Fru6P concentration, the effect of PFK-2 on ATP was a double curve. at 25°C, the Km value was 5.0×10^{-5} mol/L. As the constant Fru6P concentration declined, the Km value for the effect of PFK-2 on the ATP increased.

4. Effect of pH on PFK-2

The buffering solution used in this part of the experiment was a mixture of Bis-Tris and glycine, each at a concentration of 50 mmol/L. Either sodium hydroxide or hydrochloric acid was used to adjust the pH, and the enzyme activity set temperature was 18°C.

Figure 4 shows the tested activity under laboratory conditions when the pH returned to 7.5 after the PFK-2 had been maintained at temperature for 30 minutes in buffering solutions of different pH's. The table shows that at a pH of between 6.5 and 10.5, the PFK-2 was fairly stable. Under the conditions that we selected for this portion of the experiment, activity was basically sustained without change.

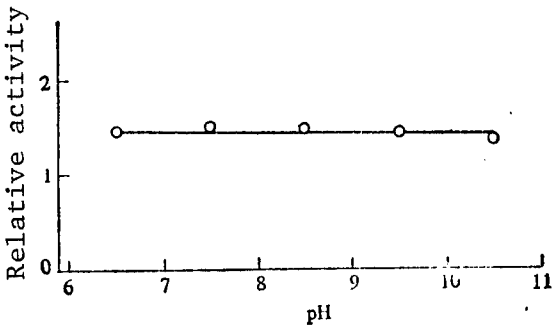

Figure 4. PFK-2 Stability at Different pH's

The concentration of regular Fru6P was 2 mmol/L. Km values and maximum reaction speeds (V_{max}) were obtained for the PFK-2 at various pH's (Figures 5 and 6). The PFK-2 was most active at a pH of 9.5.

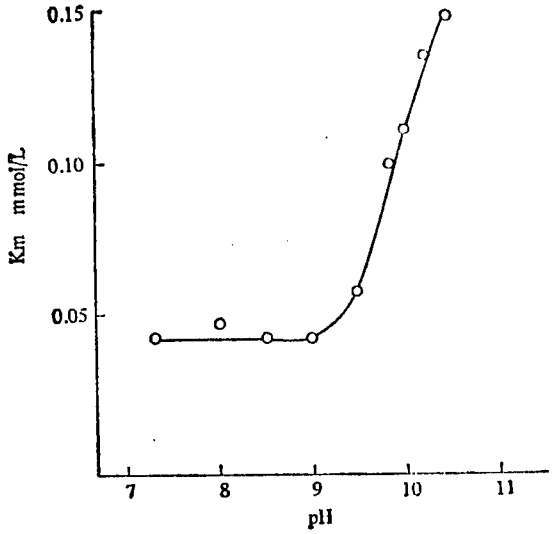

Figure 5. pH effect on PFK-2 Km (ATP)

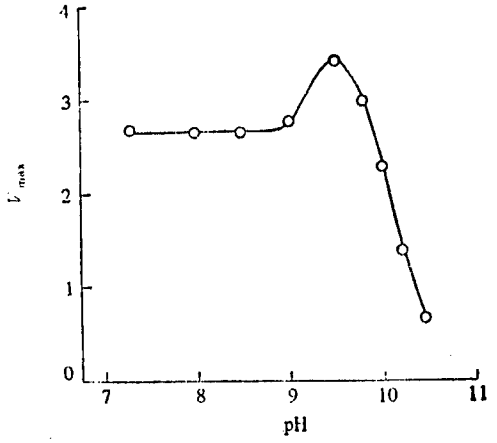

Figure 6. pH effect on PFK-2 V_{max}

Processing of data produced the diagram shown in Figure 7. Figure 7 shows that PFK-2 catalytic action required a lateral chain pKa value of 9.5 for the amino acid residue before the enzyme combined with the substrate ATP. After combining with the ATP, the value shifted to 9.8.

III. Discussion

Pilkis et al. reported on the purification and characteristics of rat liver PFK-2.[10] In the process of purifying the PFK-2 from our mouse livers, we used blue sepharose that we had made up ourselves in affinity chromatography. After eliminating PFK-1 from the preparation, we obtained excellent results. The PFK-2 and the PFK-1 may also be separated when using the gel filtering

method. However, use of the gel filtering method was limited by column capacity, and it also took a fairly long time, with the result that recovery of enzyme total activity was fairly low.

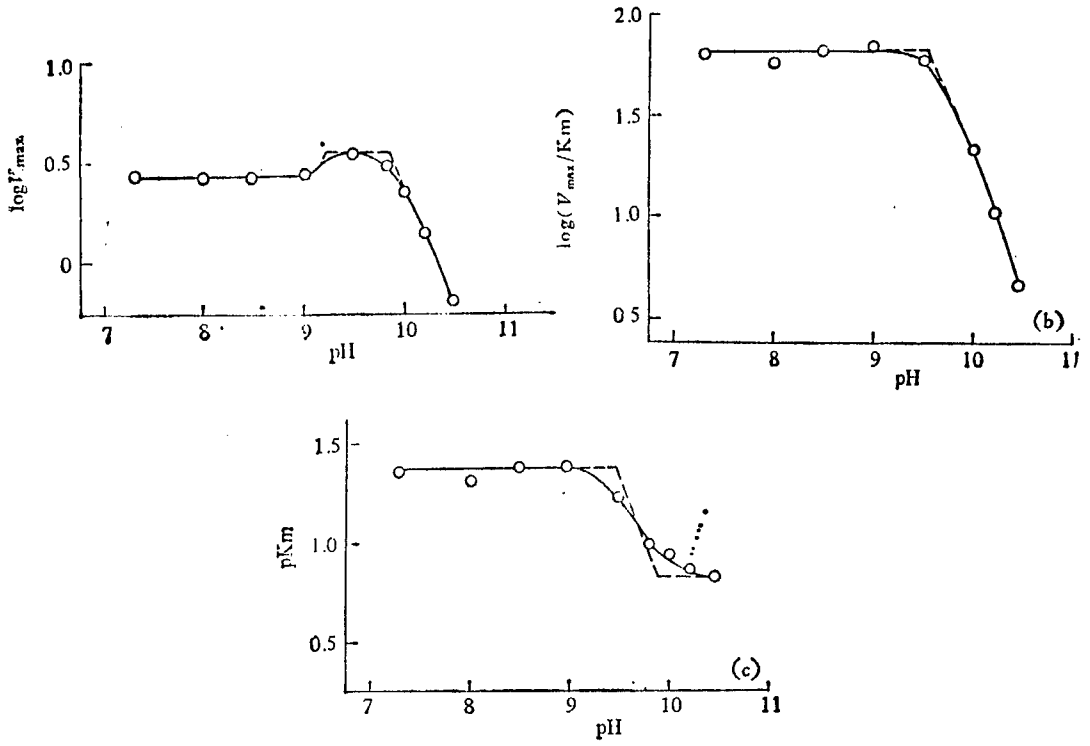

Figure 7. Functional Diagram Showing log V_{max} (a), log (V_{max}/Km) (b), and pKm (c) With Regard to pH

The PFK-2 saturation curve for Fru6P showed direct synergism, and the saturation curve for ATP was double.

The Km value set for the enzyme with regard to ATP at the regular Fru6P concentration increased as the regular Fru6P concentration declined, showing that the PFK-2 catalytic reaction may be carried out by a sequencing mechanism. Moreover, very possibly, there was a sequencing mechanism before the Fru6P was combined with the ATP.

The diagram results of a pH between 6.5 and 10.5 on the log V_{max} and the log (V_{max}/Km) of the ATP is clear. The log (V_{max}/Km) breakpoint reflects the free enzymolysis of the pKa^{ES} that has left the group, while the breakpoint for the log V_{max} reflects the enzyme and substrate combination's group-dissociated pKa^{ES}. This shows that after the enzyme combined with the ATP, the dissociated group's pKa directly changed the 0.3 pH unit. Within the pH limit, the ATP exists in a negative ion form; consequently, the pKa value's direct increase may be simply summarized as the result of a static electricity effect. Kinase and esterase frequently have Arg or Lys residue, which serve as a necessary group for substrate combination. Therefore, we

believe that the group in which the pKa is 9.5 is ε-NH_2 of Lys. The results in Figures 5 and 6 show that when the pH is greater than 9.0, both V_{max} and Km value increase. When the pH is greater than 9.5, V_{max} declines, but Km continues to increase. It seems that a pKa value that is equivalent to dissociation from the approximately 9.2 group causes an unnecessary activation. This breakpoint cannot be seen on the log (V_{max}/Km) pH diagram, possibly indicating that its correlation to the substrate ATP is not great. The nature of this residue requires further study. However, possibly its role is brought about by interaction with the Fru6P 2-hydroxylase protons, and helps the affinity attack of the oxygen atoms in the hydroxylase on the ATP γ-phosphate atoms. On the basis of the foregoing results, we imagine that the action of the PFK-2 may be carried out as shown by the Figure 8 mechanism.

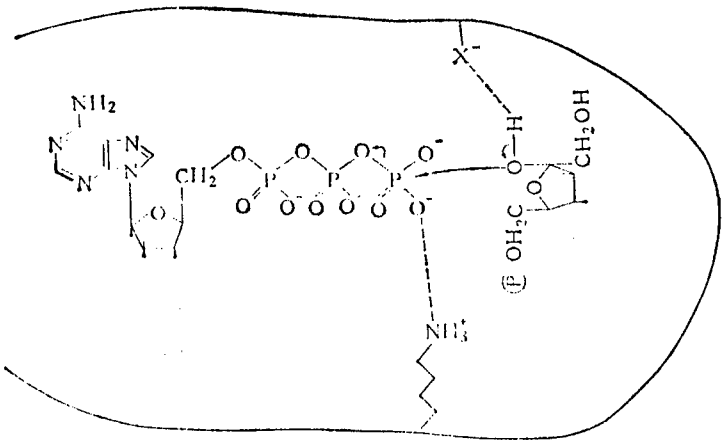

Figure 8. Imaginary PFK-2 Action Mechanism

(The $-NH_3^+$, pKa is the ε-NH_2 of the 9.5 Lys residual lateral chain, and is necessary for the enzyme to combine with the substrate ATP; $-x^-$, and an unidentified residue may interact with the 2-hydroxyl group substrate Fru6P, helping the affinity attack of the oxygen atom on the ATP γ-phosphorus atom)

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9432/6091

Expression of Vibrio Cholerae Toxin Gene in E. Coli

40081085c Beijing ZHONGGUO KEXUE [SCIENTIA SINICA, SERIES B] in Chinese No 5, May 88 pp 516-521

[Article by Ma Qingjun [7456 3237 6874], Zhou Jianguang [0719 1696 0342], Yu Xiuqin [0060 4423 3830], Liu Chuanxuan [0491 0278 2537], Xiong Lingshuang [3574 0407 7208], Xu Yongqiang [1776 3057 1730], and Huang Cuifen [7806 5050 5358], Basic Medicine Institute, Military Medical Science Academy, Beijing: "Expression in E. coli of the Vibrio Cholerae Nontoxic Enterotoxin A^-B^+ Gene"; a National Natural Sciences Fund Assisted Project]

[Text] Abstract: DNA in vitro recombinant technique was applied in the successful cloning of the Vibrio Cholerae toxin (CT) gene, reconstructing it as a CT A^-B^+ Gene in E. coli. The structured pMM-CTB recombinant plasmid was able to highly express CTB subunits in E. coli, and was able to secrete extracellularly.

Inoculations against cholera have been given for 100 years; however, it has been shown that results have not been good from the parental inoculation of dead cholera vaccine and anatoxic immunological preparations. In immunization against cholera, it is an immunization mechanism in part of the intestinal tract that plays the main role. For effective immunization protection, oral administration of an immunization preparation should be relied on in order to induce an immunity reaction from the enteromycoderm, and the generation of antibodies, principally secreted IgA.[1]

It was on this basis, and in accordance with the nucleotide sequence of the CT gene concerned, that the CT gene's recombinant plasmid pMM-CT was reconstructed into pMM-CTB. Then through the action of the E. coli lactose promoter, high expression and the extracellular secretion ability was obtained from CT enterotoxin B subunits.

I. Materials and Methods

Restriction endoclase, T_4DNA ligase, DNase I, and DNA polymerase I were all products of Biolabs Company.

Purified cholera toxin was a Sigma Company product anticholera toxin blood serum obtained by this laboratory from the immunization of rabbits with the Sigma Company's cholera toxin product.

Radioactive isotopes αP^{32}-dATP, and I^{125}-NaI were products of the Amersham Company.

1. DNA preparation and recombinant plasmid formation and analysis: Plasmid extraction, restriction enzyme enzymolysis, DNA in vitro linking and transformation, quick plasmid assay, colony hybridizing, and agarose electrophoresis molecular hybridizing were all done in accordance with reference [3].

CT gene probe preparation: CT gene recombinant plasmid pMM-CT DNA, which had been constructed by this laboratory, was double cut using EcoRI/XbaI, and a CT gene DNA segment was isolated through electrophoresis. Notch translation was used in αP^{32}-dATP marking. Radiation specific strength was 3×10^7 cpm/μgDNA.

2. Assay of toxin biological activity: Assay of Y_1 adrenal gland cells was performed in accordance with reference [7 and 8] procedures.

Rabbit intestinal loop experiment: The jejunoileum was removed from the peritoneum of a healthy rabbit weighing approximately 2 kg, and it was ligated every 5 or 6 centimeters along its length with a space of 1 centimeter between each ligated segment. Into each segment was injected 1 ml of the sample to be tested. Then the jejunoileum was replaced and the abdomen resutured. Between 14 and 16 hours later, the abdomen was reopened, and the amount of fluid retained in each segment was checked. Each centimeter of jejunoileum segment averaged an accumulation of 1.0 ml or more of fluid in a positive reaction, showing that the bacterium strain was able to produce enterotoxin.

3. Toxin antigenicity assay: Bacteria colony in situ radio-immunity was conducted in accordance with the Kemp method.[4] Cholera toxin passive immune hemolysis assay was conducted using the Serafim method,[5] and cholera toxin ELISA assay was done using the Sack method.[6]

II. Results of Experiment

1. Building of CTA^-B^+ Clone

DNA in vitro recombinant technique was applied in accordance with the applicable CT gene nucleotide sequence, placing the already constructed recombinant plasmid pMM-CT containing the CT gene in the tenth amino acid sequence of the coded CTA subunit where there was a specific XbaI site. Thus, EcoRI/XbaI enzymolysis was used to excise the CT gene's own promoter and the coded CTA subunit's initiator signal, and the N terminal's 10 amino acid sequences, separating its enzymolyzed 2.4 kb DNA segments containing the CT gene as the target gene segment for cloning. The carrier plasmid selected was pUC19, which contains a lactose (Lac) promoter, a β-galactosidase gene, and an ampicillin resistant gene. Between the Lac promoter and the β-galactosidase genes are XbaI, and EcoRI multiple clone sites. After appropriate XbaI/EcoRI enzyme cutting, its 2.68 kb segment was isolated, and both the isolated target gene and the carrier DNA segment were subjected to electrophoresis and eluted; extraction was done using phenol;

and precipitation was done using ethanol, each yielding 500 ng, then 800 units of T_4 ligase was used to conduct linking at between 10 and 12°C under linkage reaction conditions (Figure 1). Transferral to the E. coli RR_1 strain was done, with transferant being obtained in an ampicillin solid broth culturing dish. Colony in situ hybridizing (Figure 2 [not reproduced]), recombinant plasmid agarose gel electrophoresis molecular hybrid analysis (Figure 3 [not reproduced]) was used to screen the clone containing recombinant plasmid pMM-CT B.

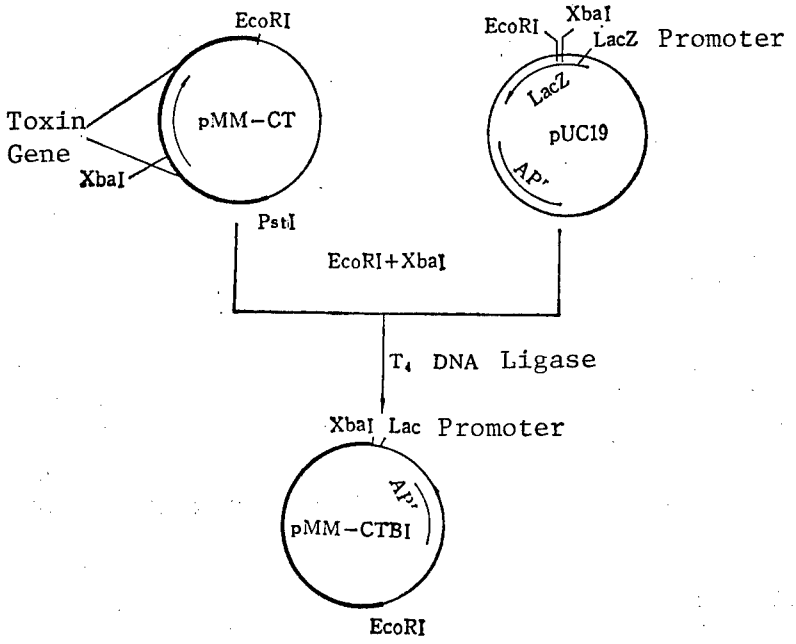

Figure 1. CTA^-B^+ Gene Clone Design

2. CT Biological Activity of the pMM-CTB Clone

As designed, the recombinant plasmid pMM-CT B that was constructed is unable to synthesize and express the toxin's toxic A subunit, and this was demonstrated in the following two experiments.

(1) Rabbit intestinal loop ligation experiment. Use of the rabbit small intestinal loop ligation experiment showed (Figure 4 [not reproduced]) the E. coli RR_1 (pMM-CTB) to be the same as the E. coli (pUC19) strain. Neither the cell-free culturing solution nor the cell cleavage extract was able to cause an accumulation of fluid in the peritoneum (0.3 ml/cm), but the Vibrio cholerae 569 B strain and the RRI (pMM-CT) E. coli were both able to cause the accumulation of fluid (1.5-2.0 ml/cm).

(2) Assay of the Y_1 adrenal gland cells. Y_1 adrenal gland cells are very sensitive to toxins. A pg quantity of toxin can cause the cells to produce morphologically varied pathological changes. Results of the assay (Figure 5 [not reproduced]) show the RRI (pMM-CTB) strain's cell-free culturing solution

and the cell cleavage extract as not being toxic to the Y_1 cells, and no morphological changes. However, the parent RRI (pMM-CT) strain that had not yet been genetically modified was toxic, causing morphological variations in the Y_1 cells.

3. CT Antigenicity of the pMM-CTB Clone

Inasmuch as the constructed recombinant plasmid pMM-CTB retained the nucleotide combination site of the coded CTB subunit gene as the initiator signal ATG, when turned on by the Lac promoter, this plasmid is able to synthesize CT's B subunit, and it possesses CT antigenicity.

(1) Colony in situ radio-immunity analysis. Protein from split open bacteria colonies containing the pMM-CTB recombinant plasmid were transferred to anti-CT serum-soaked bromacyanogen-activated filter paper. After interacting with the ^{125}I-anti-CT serum, as with the Vibrio cholerae 569 B strain, the audioradiogram showed black imprints. However, the E. coli containing pUC19 plasmids showed no black imprints, showing the pMM-CTB clone as able to express CT antigens.

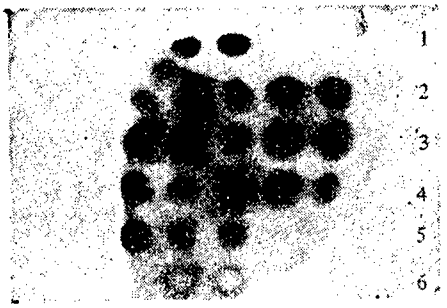

Figure 6. Colony in Situ Radio-Immune Analysis

(1: Vibrio cholerae 569 B; 2-5: E. coli RRI (pMM-CTB); and 6: E. coli JM105(pUC19))

(2) Passive immune hemolysis experiment. A passive immune hemolysis experiment was done on the cell-free culturing solution of the RR_1(pMM-CTB) strain culture, and on a sample of the cell cleavage extract in the performance of a CTB subunit antigen assay. Results (Figure 7) showed that pure toxin (Sigma Company) 100 at 2.5 ng/ml could completely hemolyze sheep red blood corpuscles, and 1 ng/ml caused a partial hemolysis. Using this as a yardstick, the CTB subunit antigen produced by RR_1(pMM-CTB) was approximately 500 ng/ml in the in vitro culturing solution, and approximately 125 ng/ml in cells.

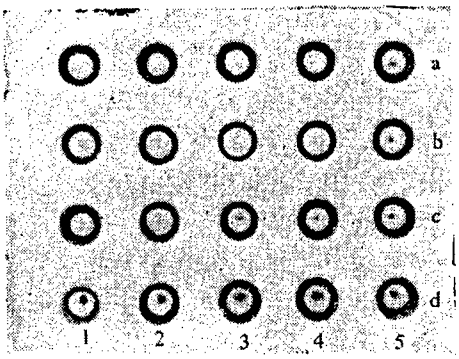

Figure 7. CT Antigen Passive Immune Hemolysis Experiment

(a: pure cholera toxin; 1-5: 100, 10, 5, 2.5, and 1 ng/ml in each pore; b: E. coli RRI(pMM-CTB) cell-free culturing solution; 1-5: dilutions were 1/10, 1/50, 1/100, 1/200, and 1/500 respectively; c: E. coli RRI(pMM-CTB) cell extract; 1-5: dilutions were 1/10, 1/50, 1/100, 1/200, and 1/500 respectively; d: CT antiserum inhibiting experiment used; 1-3: pure cholera toxin; and 4-6: E. coli RRI(pMM-CTB)

(3) GM_1-ELISA determination: A ganglioside-enzyme linked immunoassay analysis was done on the RRI(pMM-CTB) bacteria strain culture's cell-free culturing solution, and on the cell cleavage extract to make a CTB subunit antigen determination (Figure 8 [not reproduced]). Using pure toxin from the Sigma Company as the standard, the extracellular culturing solution contained approximately 500 ng/ml of B subunit antigen, and the cell cleave extract contained approximately 25 ng/ml. When IPTG induction was used, output could be increased reaching 1,000 ng/ml in the extracellular culturing solution, and 100 ng/ml in the cells.

4. Immunogenicity of the CTB Subunit Produced by the pMM-CTB Clone

The B subunit antigens produced by the pMM-CTB strain were used to immunize rabbits either through muscular injection or orally. Freund's incomplete adjuvant was used in the muscular injections. In the oral immunization, 10 ml 0.1 mol/L of $NaHCO_3$ solution was perfused before perfusion of the antigen. Basic immunization was done three times, 10 days between each one, dosages being 5, 2, and 5 μg. Thirty days after the final one, 2 μg of antigen was given to increase immunity. Seven days following immunization, aggressive intestinal coil ligation was done using the pure toxin. The results (Figure 9) showing a certain amount of protection was produced, the 5 to 6 cm intestinal segments being able to withstand an attack of between 10 and 50 ng.

III. Discussion

Since the beginning of the 1980's, very great advances have been made internationally in the application of gene engineering techniques to the study of cholera toxin gene molecular genetics and to the construction of engineered vaccines.[11-13]

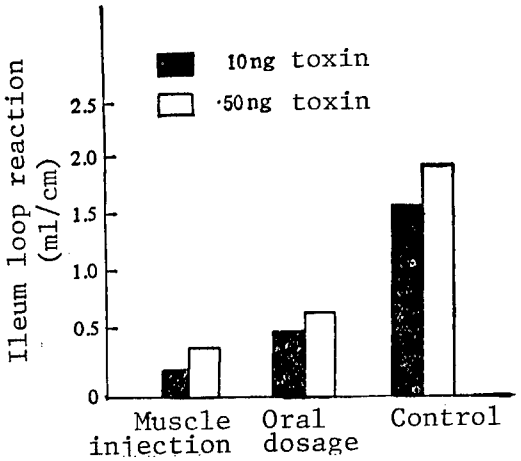

Figure 9. Immunogenicity of CT-B Subunits Produced by Engineered Bacteria

In order to construct a clone of the cholera toxin gene $A^{-}B^{+}$, we designed an E. coli lactose promoter to take the place of the cholera toxin gene's own promoter, and we eliminated the coded toxin gene A subunits' initiator signal sequence ATG, as well as the N terminal's 10 amino acid sequences. Thus, when the lactose promoter switched on, the coded toxin's A subunit gene could not produce toxicity expressing A subunit polypeptides. However, the design retained the coded B subunit gene's ribosome binding site and the initiator signal sequence. Thus, it was able to synthesize the toxin's B unit polypeptides. Results obtained from the experiment fully demonstrated this to have been a successful design, and markedly superior to both the gene frameshift and the partial A subunit gene deletion of the reference [11-13] designs: 1) The toxin B subunit polypeptides obtained through this design may be excreted outside the cells. 2) Output is fairly high. The B subunits produced by E. coli expression run between 10 to 80 times higher than those reported in references [11-13].

Though the E. coli are able to secrete various kinds of proteins to their exoplasm and adventitia, they secrete extremely little outside the cells. It is of very great significance that in our experiment the E. coli were able to secrete the cholera toxin B subunits outside the cells, showing that the E. coli are able to differentiate the cholera toxin polypeptides' secretion signals, while also being able to process them and send the B subunits outside the membranes. It is also interesting that the E. coli were unable to secrete outside the cells the E. coli heat-labile enterotoxins, but were able to secrete the CTB subunits outside the cells. It appears that the secretion outside the cells of proteins is not only related to the secretion signal peptides, but also may involve other mechanisms and is related to structural protein components and the size of molecules.

Cholera toxin B subunits possess fairly powerful antigenicity and immunogenicity; consequently, the pMM-CTB recombinant plasmid clone that we constructed in vitro might conveniently provide B subunit antigens to lay a foundation for the building of effective immunity preparations or live vaccines.

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Mutagenicity and Teratogenicity of Chlorpromazine and Scopolamine

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[Text] Mutagenicity and teratogenicity of chlorpromazine and scopolamine with indicators of chromosomal aberration, sister chromatid exchange (SCE) and teratogenesis test of gestation in mice are presented in this paper. Chlorpromazine and scopolamine were divided into five (0.25, 0.5, 1.0, 1.5 and 2 μg/ml) and six (0.5, 1.0, 2.0, 5.0, 10 and 20 μg/ml) dosage groups respectively. Each was added directly to peripheral lymphocyte cultures of 10 healthy adults. Cultures treated with saline and mitomycin served as normal and positive controls. The chromosomal aberration and SCE were simultaneously studied. The frequency of chromosomal aberration and SCE in the chlorpromazine groups were markedly higher than these of the normal controls ($p < 0.01$ or $p < 0.05$). Those of the scopolamine groups were also significantly higher. The results showed that chlorpromazine damages DNA. Usual clinical doses of scopolamine do not damage DNA, but large doses do. Using 1/50 and 1/10 of the LD_{50} dose (i.e., 1.8 and 9.2 μg of chlorpromazine or 4.6 and 59 μg of scopolamine per gram of body weight), their teratogenicity was tested in mice. Saline and cod-liver oil treated mice were used as normal and positive controls respectively. The incidences of malformed fetuses were 38.5 percent and 42.7 percent with chlorpromazine or 3.8 percent and 8.2 percent with scopolamine, while those in normal and positive controls were 0 and 28.6 percent respectively. The results showed that the teratogenic effect of chlorpromazine in mice was greater than that of scopolamine.

With progress in study of mutagenicity, teratogenicity and carcinogenicity of some chemicals during recent years, their effects on cell-genetics has been assessed.[1-3] According to some foreign reports, the majority of conventional anesthetics are not mutagenic, the toxicity of some has not been confirmed but others have stronger mutagenic effects.[4-10,14] The harm of mutagens is mainly damage to genetic material (DNA). Mutagenicity may occur at the somatic cell or the genital cell. When mutation of genital cell DNA takes place, it may result in stillbirth, barrenness and malformation. When DNA mutation occurs

in the somatic cell, it may give rise to tumor.[13-16] So screening of anesthetics is of great importance to eugenics and preventive medicine. DNA damage by the majority of mutagens can result in abnormality of chromosomal construction and sister chromatid exchange (SCE), The cell-genetics technic is a sensitive one for calculating the index of this damage.[11,12,18,19] We preliminarily screened intravenous anesthetics and found that the chromosomal aberration rate of chlorpromazine and scopolamine was significantly higher than that of controls.[17] In order to explore their mutagenic effect, we tested different doses to find the degrees of mutagenicity by using the two indices of chromosomal aberration and SCE rate. We also performed teratogenic tests on gestating mice.

Material and Methods

Mutagenic test. According to the dosage, the chlorpromazine group was subdivided into five groups (0.25, 0.5, 1.0, 1.5 and 2 μg/ml), and the scopolamine group into six groups (0.5, 1.0, 2.0, 5.0, 10 and 20 μg/ml). The dosage was appropriate to therapeutic needs of adults.

Fourteen and 16 portions of the medium containing anticoagulant blood (0.3 ml each) taken from the subjects were divided into these tested for chromosomal aberration and SCE. After the medium was cultured for 24 hours, BudR 5 μg/ml was added to the SCE specimens. After 48 hours, experimental drugs were added to the aberration medium and SCE according to the dosage group. Saline was added to the free control group and mitomycin to the positive control group. After 68 hours, the media were treated with colchicine. After 72 hours, the chromosomal aberration and SCE specimens were made by the chromosomal aberration routine technic and BudR-Giemsa air-dry method respectively.

In each chromosomal aberration specimen, 100 metaphases were examined, and in each SCE specimen the SCEs were examined for the number and percentage of chromosomal aberrations (CHAP) by the single blind method. From these, the SCE percentage (SCEP) was calculated.

Teratogenic test of mouse gestation. 3-month-old female experimental mice were used. After the mice had been isolated and raised for a month, the mice weighing above 20 g were selected. Then two female and one male mice mated in cages under the same conditions. On the second day, vaginal secretion smears were taken and observed microscopically. If spermatozoa were found, the day was regarded as zero day of gestation. Gestational mice were taken from the cage and divided into six groups by the draw lots method. The six groups were allocated to low and high chlorpromazine doses (1.8 and 9.2 μg/g mouse weight) and scopolamine (4.6 and 59 μg/g mouse weight), the free control (saline 0.3 ml) and the positive control groups (0.3 ml of cod-liver oil containing vitamins A and D 1500 u). Groups of 10 mice each were isolated and raised. The dose was calculated on the basis of the equivalent human dose rate per body surface area and made up into doses of 0.3 ml. These were injected into the abdominal cavity once daily from the sixth to the sixteenth day of gestation (cod-liver oil was given only 3 days). The mice were sacrificed and the fetuses kept for examination 2 or 3 days before parturition. The average growth weight, increased body weight and the fetal malformations were recorded.

Results

Mutagenic effects. Mean Percentage of chromosomal aberration and SCE of each dosage group.[6,8] The two indices of each chlorpromazine group were significantly higher than those of the corresponding free control group (CHAP: $p<0.01$, CSEP: $p<0.05$ or 0.01). The percentage of chromosomal aberration and SCE at progressively increasing dosage groups of scopolamine were significantly higher than those of the free control group ($p<0.01$ or 0.05), but there was no difference between the first dosage group and the corresponding free control group ($p>0.05$). The positive control group percentages were significantly higher than those of each corresponding dosage group ($p<0.0$, Tables 1, 2).

Table 1. Chromosomal Aberration and SCE Induced by Chlorpromazine at Five Doses (10 subjects)

Dosage group (ug/ml)	CHAP		SCEP	
	Metaphose	M±SD	SCEs	M±SD
0.25	981	12.8± 3.0	221	10.0±2.8
0.50	810	24.9±16.8	257	10.4±1.1
1.00	1 060	24.4±17.4	284	12.6±2.1
1.50	853	18.7±10.5	253	11.7±2.8
2.00	727	21.0±12.0	266	10.7±3.4
Free control	1 000	4.3± 1.8	315	7.8±2.3
Positive control	1 000	53.9±31.3	136	29.1±19.0

Teratogenic effect. Body weight calculation. Increased body weight of maternal mice was calculated by the body weight of the maternal mouse when killed-body weight on gestational day zero-uterus weight (containing mouse fetus) when killed.[15] Negative values showed decreased body weight. Increased body weights of maternal mice were significantly higher in every experimental group than in the positive control group ($p<0.01$) except for the low dose chlorpromazine group (Table 3).

Growth of mice fetuses. The mice fetuses that had head, front legs and hind legs formed and weight above 1.1 g were regarded as normal fetuses (all were alive). The mice fetuses whose heads and legs could be identified but the body weight was each below 1 g were regarded as abnormal fetuses (all were dead), and those whose heads and legs could not be identified and the body weight each was under 0.1 g were regarded as embryos. Embryos arrested at the blastoderm stage were regarded as absorbed. When there were traces of nidation, a blastoderm was not found and the uterus was dark-red and thickened, this was regarded as nidation. According to X^2 test, the incidence of abnormal mice fetuses was significantly lower in the free control group than in the other 5 groups ($p<0.01$, Table 4).

Table 2. Chromosomal Aberration and SCE Induced by Scopolamine

Dosage group (ug/ml)	CHAP		SCEP	
	Metaphose	M±SD	SCEs	M±SD
0.5	955	4.0± 1.6	264	8.7±2.7
1	945	10.1± 3.8	282	10.2±3.8
2	800	10.1± 2.9	288	11.5±4.0
3	880	13.8± 8.6	299	10.3±3.5
10	810	18.5±10.9	298	10.0±2.3
20	935	14.7± 8.1	270	12.9±5.0
Free control	930	4.1± 1.5	251	8.2±2.3
Positive control	1 000	53.9±31.3	136	29.1±19.0

Table 3. Increasing Body Weight in Teratogenic Test (mean±SD)

Groups	0 day	Before sacrificing	Uterine weight (contained fetus)	Increasing body weight of gravid mouse	
				M±SD	Comparison with free control group
Free control	25.3±4.8	38.4±8.4	9.2±4.9	3.9±3.3	
Positive control	26.8±2.6	24.6±5.0	1.4±1.0	-4.3±3.6	P < 0.01
LC	25.3±4.1	30.0±7.2	4.1±3.8	0.6±2.0	P < 0.01
HC	25.3±4.1	30.1±4.9	2.5±1.8	2.3±2.2	P > 0.05
LS	25.7±4.6	33.8±4.2	2.8±1.6	5.3±4.4	P > 0.05
HS	26.1±4.2	32.6±5.4	3.7±2.4	2.8±2.7	P > 0.05

Abbreviations: LC = Low dose chlorpromazine, LS = Low dose scopolamine, HC = High dose chlorpromazine, HS = High dose scopolamine.

Table 4. Fetal Mouse Growth in Each Group

Groups	Total fetus number	Normal fetuses		Abnormal fetus	Abnormal fetuses			
		No.	%		Embryo	Absorbed	Nidation	%
Free control	59	49	83.1	4	0	6	0	16.1
Positive control	63	0	0	7	5	33	18	100.0
LC	60	8	13.3	40	0	12	0	86.7
HC	63	0	0	39	9	9	6	100.0
LS	60	0	0	53	7	0	0	100.0
HS	68	0	0	49	0	19	0	100.0

Mean body weight of mice fetuses. The mean body weight of the mice fetuses whose heads and legs could be identified were calculated; it was significantly higher in the free control group than in the other groups ($p > 0.05$), Table 5).

Mice fetal malformations. Malformations of mice fetuses (normal and abnormal) whose heads and legs could be identified in each group were observed by naked eye. No malformations were found in the free control group. The malformation rate of mice fetuses was markedly high in the other groups. The rate was significantly higher in the positive control group, and in the low and high dose groups of chlorpromazine than in the scopolamine dosage group, $p < 0.01$, by X^2-test (Table 6).

Table 5. Mean Body Weight of Fetal Mice of Each Group (Normal Fetuses, Abnormal Fetuses and Embryos)

Groups	Gravid mice	Mice	Mean body weight of fetal mice (g/mouse)
Free control	9	53	1.5±0.4
Positive control	2	12	0.4±0.3
LC	8	48	0.5±0.4
HC	8	48	0.3±0.2
LS	10	60	0.4±0.3
HS	7	49	0.3±0.1

Discussion

The effect of drugs on the genetic construction of somatic cells and genital cells of humans is increasingly receiving our attention. Some drugs can cause mutagenesis at varied levels and involve a tiny part of a chromasome and single gene. These can damage a whole segment of a chromasome that contains some genes or a whole chromosomal group.[20] Changes in chromosomal groups can be sensitively detected by the cell-genetics technic, and lymphocyte changes in peripheral venous blood can directly reflect human somatic cell mutation. Chemicals inducing chromosomal aberration can also induce SCE. Sensitivity of damaged DNA, reflected by SCE is much higher than that reflected by chromosomal aberration. Since DNA damage and SCE are highly correlated with mutagenic effect, they are important indices in detecting the mutagenic effects of chemicals. These results suggest that both indices are significantly higher in the five dosage groups of chlorpromazine than in the free control group ($p < 0.001$), even though the first dose (0.25 μg/ml of culture solution) is 10 times lower than the clinical dose. It is evident that chlorpromazine damage of DNA is obvious. Both indices show no increase when scopolamine is given within the limits of the clinical therapeutic dose. They are markedly

Table 6. Percentage of Malformed Fetal Mice

Groups	Fetal mice	Type of malformation				Percentage of deformed fetuses
		Eyes open	Deformed toes	Deformed limbs and trunk shortage	Cephalocele	
Free control	53	0	0	0	0	00.0
Positive control	7	7	0	0	1	28.6
LC	48	41	33	0	0	38.5
HC	39	36	21	10	0	42.9
LS	53	0	0	8	0	3.8
HS	49	11	0	5	0	8.2

higher in progressively increasing dosage groups than in the free control group ($p<0.01$) and the first dosage group (0.5 μg/ml of culture solution, $p<0.05$), suggesting that increased scopolamine damages DNA and changes chromosomal construction. Because there is very high correlativity between mutagenicity, teratogenicity and cancerogenicity of the chemicals[18,19,21] potential teratogenic harm can be judged by detecting mutagenicity. In order to further confirm the final morphological effect of the mentioned drug mutagenicity on embryo growth and organ formation as well as its effect on the body, teratogenic tests were performed in mice using low dosage group (1/50 of LD_{50} and high dosage group 1/10 of LD_{50}). The percentage of malformed fetuses was 38.3 percent in the LD and 42.9 percent in the HD groups of chlorpromazine, 3.8 percent in the LD and 8.2 percent in the HD groups of scopolamine, 0 percent in the free control group and 28.6 percent in the positive control group respectively. The test results showed obviously that both drugs can hamper fetal growth in the mouse, and that the teratogenic effect was more evident with chlorpromazine than with scopolamine. Although the mentioned results were positive, the experiment should be repeated a number of times as the differences between animals and humans may vary a great deal in regard to race, strain, and individual characteristics. If the same results are produced in experiments on diverse kinds of animals and high mammals, deductions can then be made about humans.[22] However, the mutagenic and teratogenic effects noted suggest that the two drugs should be administered with caution to gravid patients, particularly at the stage of organ formation 3-6 weeks after gestation.

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/08309

Plasmid DNA Survey of Clinically Isolated Shigella Strains in Shanghai Area

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[Text] Plasmids and biological characteristics of Shigella-strains isolated in Shanghai were studied for comparison with those of strains isolated in Tokyo from travellers back from various areas of South East Asia. Invasive ability was confirmed to be related to 140 or 120 mega dalton (M) plasmids. Thirty to 70 M plasmids were found to be conjugative R plasmids. Incidences of ampicillin, trimethoprim and mercury resistant strains (R plasmids) were higher in Shanghai strains than in South East Asian strains.

A variety of smal plasmids were detected in all strains. In S flexneri strains, 2.0 and 2.6 M plasmids were found to be common in the strains from both areas, and in Sonnei strains, 3.4 M col El plasmids were common. The plasmid patterns formed by the small plasmid combinations were not always specific for species or serotype. But at least these small plasmid DNA patterns could be used to trace epidemic strains.

We have been collecting Shigella strains isolated from dysentery patients in various areas of Asia. At first, the strains were collected from isolation hospitals for infectious diseases in Tokyo, because recently there have been increasing numbers of dysentery patients in these hospitals, although we have almost no endemic cases of dysentery. Some travellers back from South East Asia have been found to be infected, and the strains from travellers from different areas may represent the endemic strains in the area.

Plasmid analysis of these strains revealed the presence of various small plasmids as well as large plasmids responsible for invasive character and antibiotic resistance. Certain small plasmids seem to be related to certain species and types and overall patterns of these small plasmids seem to be useful for typing epidemic strains.[1]

To confirm these observations, we should further examine plasmids of Shigella strains from different areas. To fulfil this requirement, we surveyed plasmids of clinically isolated Shigella strains in Shanghai area, and compared them with those of South East Asia.

Material and Methods

Strains employed. Fifteen strains of S flexneri and 17 strains of S sonnei were collected from strains isolated from patients of different 1985 endemics in Huashan Hospital, and 6 strains of S flexneri and 4 strains of S sonnei isolated in the Shanghai Hygiene and Antiepidemic Center during the same period were used. These strains were repurified on Congo red (final 0.0015 percent)-containing galactose (final 0.5 percent) nutrient agar (Eiken Chemical, Tokyo) plates and dye-accumulating colonies were selected and stored at -80C. Escherichia coli K-12; RC85 nal was used as a recipient strain for conjugative R Plasmids.[1] S flexneri 2457-0[2] and E coli V517[3] were used to supply marker plasmids.

Antibiotic susceptibility. Drug susceptibility was estimated by the minimal inhibitory concentrations (MIC) determined by the standard method of the Japanese Society of Chemotherapy.[4] MIC of sulfamonomethoxine (SA, Daiichi Seiyaku, Tokyo) streptomycin sulfate (SM, Meiji Seika, Tokyo), kanamycin sulfate (KM, Meiji Seika), tetracycline hydrochloride (TC, Japan Lederle, Tokyo), chloramphenico (CP, Sankyo, Tokyo), sodium ampicillin (AP, Banyu Seiyaku, Tokyo), nalidixic acid (NA, Daiichi Seiyaku) trimethoprim (TP, Sigma, St Louis) and mercuric chloride (Hg, Wako Pure Chemical, Osaka) were measured. MIC distributions were figured and strains not belonging to the lowest group were judged as resistant strains.

Colicin production. Colicin production was estimated by their growth inhibition to E coli Row and typed by growth inhibition patterns to E coli Row/B, Row/IV, Row/E and Row/K and Salmonella typhimurium LT-2. Col E producers were subtyped by their growth inhibition patterns to E coli K-12; W3102 (col E1), W3102 (col E2) and W3102 (col . Test strains were cultured on nutrient agar plates and killed by chloroform vapor. Three ml of soft agar containing the exponentially growing indicator strain was poured onto the plates, and cultured at 37C overnight. Colicin producers were judged by the presence of growth inhibition zone around the test cell culture.

Detection of conjugative R plasmid. Each mid-exponentially growing culture of the resistant strain in Bacto-penassay broth (Difco, Detroit) was fixed with an equal volume of E coli RC85 nal at 37C. After two-hour incubation, they were plated onto the bromtymol-blue (BTB) lactose agar plates containing nalidixic acid and one of the mentioned drugs with the exception of SA, TP and Hg, and cultured at 37C for two nights. Concentrations of SM, KM, TC, CP, AP and NA added to the selective plates were 12.5, 25, 25, 25, 50 µg/ml, respectively. Transconjugants grown on the selective plates were purified on the same selective agar plates and tested for unselected resistance by spotting on sensitivity disc agar N (modified Mueller Hinon agar Nissui Seiyaku, Tokyo) plates containing one of the drugs at the mentioned concentrations. SA, TP and Hg were added to the medium at 200, 50 and 200 µg/ml, respectively.

Gut epithelial cell invasive ability. Monolayer HeLa S3 cells cultured in Eagle's minimal essential medium (MEM, antibiotic free, Nissui Seiyaku) containing 5 percent fetal bovine serum (FBS, Gibco, Grand Island, N.T.) were suspended in the same medium at a cell density of 10^4 cells/ml after trypsinization (0.25 percent solution, Difco), and each 0.4 ml of the suspension was dispensed into Lab-Tek tissue culture chamber/slides (8 chamber, Miles lab., Elkhart, Indiana) and cultured at 37C for 48 hours in CO_2 incubator.

Shigella strains selected on Congo red agar were cultured in brain heart infusion broth (Difco) at 37C overnight. Five µl of bacterial culture (about 10^7 cells) was inoculated into a chamber and incubated at 37C for two hours. HeLa cells were washed three times with MEM containing 3 percent FBS and 50 µg/ml of KM and cultured another 6 hours in the same medium. After removing the chamber septa the slides were washed gently with phosphate buffer saline (PBS), fixed with methanol and stained in 2 percent Giemsa solution at 37C for an hour. Strains grown in HeLa cell cytoplasm were judged positive.

Detection of plasmid DNA. The method used to identify plasmid DNA was principally the same as the method described by Kado and Liu.[5] Five µl of loading buffer composed of 60 percent glycerol, 40 mM Tris-acetate buffer (pH 8.3), 40 mM EDTA, 0.3 percent bromophenol blue, 0.3 percent xylene cyanol and 200 µg/ml of ANase was added to 20 µl of the sample and applied on 0.5, 0.7, and 1.0 percent agarose gels in 40 mM Trisacetate buffer (pH 8.3) with 2 mM EDTA. After electrophoresis, the gel was stained with ethidium bromide and rinsed twice with water and photographed under UV light. Molecular sizes of plasmids were estimated by their migration distances and compared with those of the standard plasmids from S flexneri 2457-0 and E coli V517.

Results

Plasmid DNAs detected in the 0.5, 0.7 and 1.0 percent agarose gels are shown in Figs 1-3. Drug resistance, invasive ability, colicin production and plasmid patterns of Shigella strains from Huashan Hospital and from Shanghai Hygiene and Antiepidemic Center are summarized in Tables 1, 2. Only one of 42 strains was sensitive to all drugs tested. No nalidixic acid resistant strains were found. The combined resistance to SA, SM, TC, CP and TP was the most prevalent pattern of the strains (R plasmids as well). Incidence of TP resistant strains was higher in S sonnei than in S flexneri. Only two strains were resistant to KM. Mercury resistant strains were only found in the Huashan Hospital isolated strains. Thirty to 70 megadalton (M) plasmids were detected from transconjugants. All resistance markers were found in a unit in the transconjugants, although some of the transconjugants from the same isolated strain lacked some resistance markers. Molecular sizes of conjugative R plasmids from S flexneri seemed to be different from those from S sonnei. The most prevalent R plasmids from S flexneri were 60 M, but those from S sonnei were 36 M.

Most of the strains were invasive, and these strains of S flexneri and S sonnei had 140 and 120 M plasmids, respectively. Six of 8 non-invasive strains were found to have shorter size plasmids of 80 to 120 M, and another two strains had no large plasmids. Even in some of the invasive strains, shorter plasmids of 70 to 115 M were found.

Table 1. Drug Resistance, Invasive Ability and Plasmids of Shigella Strains Isolated in Huashan Hospital, Shanghai Medical University

No.		Species	Resistance patterns	Cell invasion	Plasmids molecular weight (×M daltons)
F85-	1	S. flexneri 1a	SA SM TC CP AP	+++	140 60 2.0 1.8 1.0
	5		SA SM TC CP	+	140 110 2.6 2.4 2.2 2.0 1.0
	6		SA SM TC CP AP Hg	−	110 2.6 2.0 1.0
	10		SA SM TC CP	++	140 3.7 2.0 1.8 1.0
	11		SA SM TC CP	++	140 3.7 2.0 1.8 1.0
	17	S. flexneri 1b	SA TC CP TP	+	140 30 2.6 2.0 1.0
	3	S. flexneri 2a	SA SM TC CP AP TP Hg	+++	140 60 6.5 4.8 3.6 2.0
	8		SA SM TC CP AP TP	+++	140 60 6.5 4.8 2.6 2.0
	9		SA SM TC CP AP TP	+++	140 70 6.5 4.8 2.6 2.0
	15		SA SM TC CP AP TP Hg	+++	140 70 60 3.7 2.6 2.0
	16		SA SM TC CP AP TP Hg	+	140 70 2.6 2.0 1.4
	18		SA SM CP AP TP	+++	140 60 6.5 2.8 2.6 2.0 1.0
	7	S. flexneri 2b	SA SM TC CP TP Hg	+++	140 60 50 2.6 2.4 2.0 1.0
	2	S. flexneri 3a	SA SM TC CP AP	+++	140 70 36 30 2.6 2.0
	14		SA SM TC CP	+++	140 70 8.0 5.8 4.0 3.4 2.0
S85-	1	S. sonnei	SA TC CP AP TP	+++	120 80 3.4
	2		SA SM TC CP AP TP	++	120 80 36 3.4
	3		SA SM TC CP AP TP	++	120 115 8.0 3.4
	4		SA TC CP AP TP Hg	++	120 80 30 3.4
	5		SA TC CP AP TP Hg	+	120 80 3.4
	6		SA TC CP AP TP Hg	+	120 80 8.0 3.4
	7		SA TC CP AP TP Hg	+	120 80 3.4
	8		SA SM TC CP AP TP Hg	+	120 80 36 3.4
	9		SA SM TC CP AP TP	+	120 80 36 3.4
	10		SA SM TC CP TP Hg	−	100 80 6.5 3.9 3.7
	11		SA TC CP AP TP Hg	+	120 80 8.0 3.4
	12		Sensitive	++	120 8.0 3.4
	13		SA SM TC CP AP TP	−	80 8.0 3.4 2.6
	14		SA SM TC CP AP TP KM	+	120 100 36 3.4
	15		SA SM TC CP AP TP	+	120 100 36 3.4
	16		SA SM TC CP AP TP	+++	120 70 36 4.0 2.3
	17		SA TP Hg	−	90 40 3.4

SA: sulfamonomethoxine, SM: streptomycin sulfate, TC: tetracycline-HCl, CP: chloramphenicol, AP: ampicillin sodium, TP: trimethoprim, KM: kanamycin sulfate, and Hg: mercury

Table 2. Drug Resistance, Invasive Ability and Plasmids of Shigella Strains Isolated in Shanghai Antiepidemic Center

No.	Species	Resistance patterns	Cell colicin invasion production		Plasmids molecular weight (× M daltons)
P113	S. flexneri 1b	SA	−	−	1.0
P272		SA SM TC CP	−	−	120 36 2.6 2.0 1.9
P167	S. flexneri 2a	SA TC CP	+++	−	140 2.6 2.0
P465		SA SM TC CP AP	+++	−	140 3.9 2.6 2.0
P582	S. flexneri 2b	SA SM TC CP AP TP	+++	−	140 60 30 2.6 2.0 1.9
P464	S. flexneri 4a	SA	++	−	140 50 6.0 3.7
P377	S. sonnei	SA SM TC CP TP KM	−	−	50 7.0
P592		SA SM TC CP AP TP	+	E	120 110 8.0 3.4
P496		SA SM	−	−	4.8 1.0
P549		SA SM TC CP TP	+++	E	120 50 30 3.4

SA: sulfamonomethoxine, SM: streptomycin sulfate, TC: tetracycline-HCl, CP: chloramphenicol, AP: ampicillin sodium, TP: trimethoprim, KM: kanamycin sulfates, and Hg: mercury

Table 3. Drug Resistance, Invasive Ability and Plasmids of Shigella Strains Isolated From Patients From Different Areas of South East Asia

No.	Species	Resistance patterns	Cell invasion	Colicin production	Plasmids molecular weight (× M daltons)
1	S. flexneri 1b	SA SM TC CP AP TP Hg	—	—	70 4.4 4.0 2.6 2.0 1.4 1.0
2		SA SM TC CP AP	—	—	8.0 3.7 2.6 2.0 1.8 1.4 1.0
3		SA SM TC CP AP TP	+	—	140 70 3.7 2.6 2.0 1.8 1.4 1.0
4		SA SM TC CP AP	+	—	140 70 3.7 2.6 2.0 1.8 1.4 1.0
5		SA SM TC CP AP	+	—	140 70 3.7 2.6 2.0 1.8 1.4 1.0
6		SA SM TC CP AP TP	+	—	140 70 3.7 2.6 2.0 1.8 1.4 1.0
7		SM TC CP	+	—	140 60 2.6 2.0 1.8 1.4 1.0
8		SA SM TC	+	—	140 4.8 4.0 3.7 2.6 1.0
9	S. flexneri 2a	SA SM TC CP AP TP	+	—	140 40 7.0 4.0 3.7 2.6 2.0
10		SM TC CP	+	—	140 4.0 2.6 2.0
11		SM TC	+	—	140 5.8 2.6 2.0
12		SA SM TC	+	—	140 25 7.0 4.0 3.8 3.4 2.6 2.0
13		SA SM	+	—	140 7.0 4.8 4.0 3.7 3.4 2.6 2.0
14	S. flexneri 2b	SA SM TC CP AP TP Hg	+	—	140 100 70 2.6 2.0 1.8 1.4 1.0
15	S. flexneri 4a	SA SM CP	+	—	140 60 6.5 3.8 2.5 1.0
16		SM	+	—	140 15 6.5 4.4 3.7 2.5 1.0
17	S. flexneri 6	TC CP	+	—	140 60 4.8 4.0 3.4 1.8
18		TC CP	+	—	140 4.8 3.8 3.7 3.4 1.8 1.0
19	S. flexneri v.X	SA	+	—	140 90 25 6.5 4.0 3.9 2.6 2.0
20	S. sonnei	SA SM TC CP AP KM TP	—	—	3.7
21		SA SM TC CP	—	E+	4.8 4.0 1.8
22		SA SM TC CP	+	E+	120 4.8 3.7 3.4 1.8 1.4
23		SA SM TC CP	—	E	5.8 3.9 3.8 3.4 1.8
24		SA SM TC CP	—	E	5.8 4.8 3.9 3.8 3.4 1.8
25		SA SM TC CP	+	E+	120 5.8 3.8 3.7 3.4 2.6 1.8
26		SA SM TC CP	+	E+	120 3.8 3.7 1.8
27		SA SM TC	+	E	120 3.4
28		SA SM TC	—	E+	5.8 4.8 3.4 1.8 1.4
29		SA SM TC	—	E+	5.8 4.8 3.4 1.8
30		SA	—	E	3.9 1.4

SA: sulfamonomethoxine, SM: streptomycin sulfate, TC: tetracycline-HCl, CP: chloramphenicol, AP: ampicillin sodium, KM: kanamycin sulfate, TP: trimethoprim, Hg: mercury.

Most of the S sonnei strains were found to produce col E1 but only one col E1 producing strain was found in S flexneri. The production of col E1 seems to be related to the presence of 3.4 M plasmids in S sonnei.

All strains had various combinations of small plasmids of 1.0 to 8.0 M. Among them, 2.0 and 2.6 M plasmids were numerous in S flexneri strains and 3.4 M plasmids in S sonnei strains. That is, 19 of 21 S flexneri strains had 2.0 M plasmids and 16 of 21 strains had both 2.0 and 2.6 M plasmids. Seventeen of 21 S sonnei strains had 3.4 M plasmids. In contrast, the 3.4 M plasmid was found in only one of 21 S flexneri strains and the 2.6 M plasmid was found in only one of 21 S sonnei strains. No 2.0 M plasmid was found in S sonnei strains.

Discussion

Drug resistance, invasive ability, colicin production and plasmid patterns of the Shigella strains isolated from patients back from various areas of South East Asia are shown in Table 3.[1] Incidences of ampicillin, trimethoprim and mercury resistant strains were far higher in Shanghai than in South East Asia. Incidences of conjugative R plasmids were also higher in Shanghai strains than in South East Asian strains, no S sonnei strains from South East Asia had any conjugative R plasmids. Molecular sizes of S flexneri R plasmids were similar in the strains from both Shanghai and South East Asia. R plasmids of S flexneri and S sonnei differed in molecular size. These results suggest that Shigella R plasmids had species specificity.

As already well documented,[2] the gut epithelial cell invasive ability of our clinically isolated Shigella strains was accompanied by 140 or 120 M plasmids and the non-invasive strains had no large plasmids or at least had reduced size plasmids. Some invasive strains also had reduced size plasmids as well as 140 or 120 M plasmid. This may partly be due to instability of the large plasmid and partly due to less effort on our part to select good clones.

In our former plasmid survey of South East Asian strains, 1.8 and 1.4 M plasmids were found in both S flexneri and S sonnei, and 1.0 M plasmids in only S flexneri. Interestingly enough, of the South East Asian strains, only S flexneri strains 2a, 6 and variant X did not have 1.0 M plasmid. But in Shanghai strains, 1.8 and 1.4 M plasmids were found only in a few S flexneri strains, none being seen in S sonnei. Only one of 8 strains of S flexneri 2a had the 1.0 M plasmid, suggesting that the 1.0 M plasmid is not type specific plasmid. Plasmid patterns of Shanghai strains were simpler than those of the South East Asian strains, especially S sonnei strains. So, plasmid patterns thought to be species and serotype specific in South East Asian strains were not so in Shanghai strains. But these small plasmid DNA patterns can be used to trace epidemic strains of Shigella.

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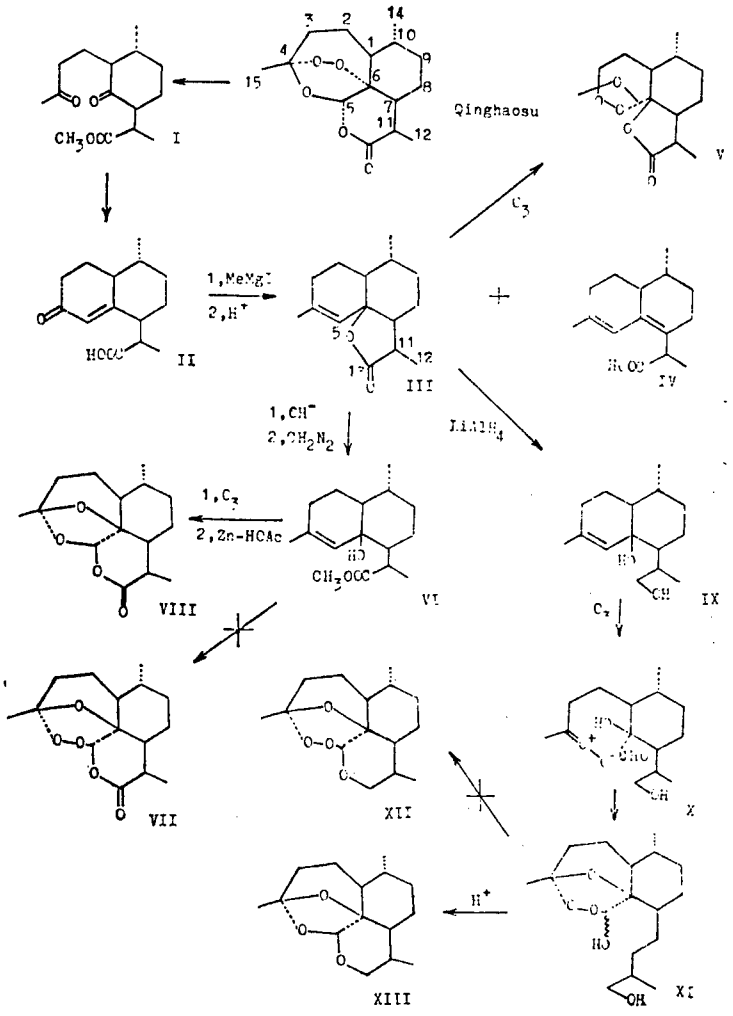
/08309

Synthesis of Qinghaosu Analogues Via Ozonization*

40091064 Beijing YAOXUE XUEBAO [ACTA PHARMACEUTICA SINICA] in Chinese Vol 23 No 6, Jun 88 pp 452-455

[English abstract of article by Zhang Jingli [1728 2529 7787], et al., of Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences]

[Text] This paper reports some synthetic efforts toward the preparation of qinghaosu analogues from a relay intermediate by means of ozonization. α, β-unsaturated ketonic acid II, a degradation product from qinghaosu, was treated with a methyl Grignard reagent at low temperatures to afford an allylic lactone III which, on ozonization, was converted to a stable ozonide V. The reduction of III by lithium aluminum hydride, followed by ozonization, gave a 1, 2, 4-trioxan compound XI, which could not be converted to a tetracyclic compound similar to qinghaosu. However, hydrolysis of III, followed by ozonization, yielded a mixture from which only an analogue of deoxyqinghaosu was obtained. (* Qinghaosu - a very effective antimalarial drug)



Scheme 1. Route of synthesis

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Expression of XylE Gene in Agrobacterium Tumefaciens--Construction of Multipurpose Cloning Vectors

40091065 Shanghai SHIYAN SHENGWU XUEBAO [ACTA BIOLOGIAE EXPERIMENTALIS SINICA] in Chinese Vol 21 No 2, Jun 88 p 147

[English abstract of article by Zhu Qun [2612 5028], et al., of Shanghai Institute of Plant Physiology, Chinese Academy of Sciences]

[Text] The XylE gene, originating from the TOL plasmid PWWO of Pseudomonas putida mt-2, encodes catechol 2, 3-dioxygenase (ED, 1.13.11.2). This enzyme catalyzes the conversion of catechol (colorless) to 2-hydroxymuconic semi-aldehyde (yellow). The expression of this gene in bacteria can be detected simply the spraying the plates with catechol, and the colonies of cells that express the XylE gene become yellow. The XylE gene of Pseudomonas putida has been expressed in Escherichia coli, Bacillus subtilis and some eukaryotic cells.

Using the structural gene of XylE and the promoter sequences of the tetracycline resistance gene of the pBR322 plasmid, the authors constructed a recombinant plasmid pBZ731. Several unique restriction endonuclease sites, i.e., EcoRI, Hind III, BamHI, HpaI and KpnI, exist in pBZ731. Since catechol is much cheaper than X-gal and IPTG, it is advantageous to use this marker for screening recombinant plasmids.

In order to facilitate the introduction of the XylE gene into Agrobacterium tumefaciens, the authors constructed another recombinant plasmid pBZ732 by inserting a kanamycin resistance gene into the plasmid pBZ731. It was found that the promoter of the tetracycline resistance gene can promote the XylE gene expression not only in E. coli, but also in A. tumefaciens. The catechol 2.3-dioxygenase activity has been detected in both bacteria.

The XylE gene has been used as a marker of animal cell transformation. It is worth studying the possibility of using this gene as a marker in the genetic transformation of plant cells.

9717

Epidemiological Study of Relationship Between Hepatitis B, Liver Cancer--Prospective Study of Development of Liver Cancer, Distribution of HBsAg Carriers, Liver Damaged Persons in Guangxi

40091066a Beijing ZHONGHUA LIUXINGBINGXUE ZAZHI [CHINESE JOURNAL OF EPIDEMIOLOGY] in Chinese Vol 9 No 4, 1988 pp 220-223

[English abstract of article by Ding Zhengrong [0002 2973 2837], et al., of the Department of Hepatitis Research, Anti-epidemic Center of Guangxi, Nanning]

[Text] A total of 22,830 persons over 20 years old were divided according to whether they were HBsAg carriers and their degree of liver damage, and were followed up for an average of 6.8 years each. The relative risk and death rate of primary liver cancer (PLC) in the HBsAg carriers with severe liver damage (hepatomegaly and biochemical abnormalities coexisting) were the highest in the areas of high and low incidence of PLC, with the relative risk being 37.3 and 22.5, respectively. The relative risk and death rate of PLC in noncarriers without liver damage were the lowest. The occurrence of PLC paralleled the degree of liver damage. The relative risk of PLC was seven times higher in HBsAg carriers than in noncarriers. The average onset of PLC in HBsAg carriers with severe liver damage was considerably earlier and at a younger age. It is suggested that the persistent HBV infections and widespread distribution of liver damage in hyperendemic areas of PLC are the factors facilitating the higher and earlier development of PLC.

9717

Study of Dog as Animal Reservoir for Hemorrhagic Fever with Renal Syndrome

40091066b Beijing ZHONGHUA LIUXINGBINGXUE ZAZHI [CHINESE JOURNAL OF EPIDEMIOLOGY] in Chinese Vol 9 No 4, 1988 pp 227-229

[English abstract of article by Zhang Yun [1728 0061], et al., of Nanjing Military Medical Institute]

[Text] From 1985-1986, 86 specimens of dog lung tissue collected from endemic areas of HFRS in Anhui and Jiangsu provinces were tested for the HFRS antigen by IFAT. Seven were found to be positive, with a positive rate of 8.1 percent. Two strains of the HFRS virus were isolated from the HFRS antigen-positive lung tissue. It was found during an epidemiological survey that the HFRS incidence rate in families raising dogs was significantly higher than that of the controls without dogs, and the apparent HFRS infection rate in healthy people who had frequent contact with dogs was significantly higher than that in people who had no contact with dogs. Evidence from these investigations shows that dogs may serve as reservoirs for the HFRS virus, and attention must be paid to dogs when attempting to control HFRS.

9717

High T_c Superconducting Materials Doped With Transition and Other Metal Cations

40090120a Changchun JILIN DAXUE ZIRAN KEXUE XUEBAO [ACTA SCIENTIARUM NATURALIUM UNIVERSITATIS JILINENSIS] in Chinese No 2, 28 May 88 pp 63-65

[Article by Su Wenhui [5685 2429 6540], Zhou Jianhua [0719 1696 0577], Liu Hongjian [0491 1347 1696], Cui Mingji [1508 2494 1213], Jin Changqing [7246 7022 7230], Li Zhanping [2621 2069 5493], Sun Changyong [1327 7022 0578], Zhuang Jinhua [5445 6930 5478], and Wang Yifeng [3769 0001 1496] of the Department of Physics, Jilin University]

[Abstract] The paper describes the preparation of superconducting materials of Y-Ba-Cu-O system doped with Bi, Sc, V, Cr, Ti, Cd, Zn, Mo, W, Fe, Co and Ni. Several factors that may be responsible for superconductivity are discussed. For specimens [$Y_{0.3}Ba_{0.7}Cu_{1-y}M_yO_{3-\delta}$, M = Bi, Cr, Ti, Cd, Zn, Mo, W, Fe, Co, Ni (y = 0.2); M = V (y = 0.2, 0.4, 0.6); M = Sc (y = 0.2, 1)] doped with Bi, Sc, V and Cr (y = 0.2), the superconductivity is similar to that of Y-Ba-Cu-O system materials. The resistance versus temperature curves, and the X-ray diffraction versus temperature curves are shown in two of three figures. The other figure shows X-ray diffraction spectra. The only table in the text lists microhardness data. References: 7, 5 in English, 1 each in Chinese and in Norwegian. The investigation was partially funded by China's State Natural Science Fund Commission.

The paper was received for publication on 20 June 1987.

10424/6091

Variations in Resistance and Structure in Sintering Process for the $YBa_2Cu_3O_x$ System

40090120b Changchun JILIN DAXUE ZIRAN KEXUE XUEBAO [ACTA SCIENTIARUM NATURALIUM UNIVERSITATIS JILINENSIS] in Chinese No 2, 28 May 88 pp 66-68

[Article by Zhang Tiechen [1728 6993 5256], Dong Weiyi [5516 4850 5030], Zhao Yongnian [6392 3057 1628], Zhang Zhilin [1728 1807 2651], Zhang Zhaojin [1728 0340 2516], Mao Zexin [3029 3419 2450], Song Bin [1345 2430], Yang Haibin [2799 3189 1755], Wang Lizhong [3769 2429 0022], and Zhou Guangtian [6760 1684 3944] of the Institute of Atomic and Molecular Physics, Jilin University; and Zhang Zhigui [1728 5268 6311] of the Chemistry Department, Jilin University]

[Abstract] A method of sintering high T_c superconducting material, $YBa_2Cu_3O_x$, is proposed. The corresponding relationship of resistance versus structure for the $YBa_2Cu_3O_x$ system in a sintering process was studied experimentally. One of two figures in the text shows the experimental arrangement. Pulverized Y_2O_3, BaO and CuO were used as the starting materials to be blended in proportions of Y:Ba:Cu = 1:2:3. The relationship between resistance and temperature is shown in the following table, the only table in the text:

Temperature in °C	25	300	350	400	450	500	550	600	700	750	800	850	900	950
Resistance in ohms	$\sim\infty$	$\times 10^3$	25.25	18.40	8.50	5.10	2.50	3.00	2.30	2.10	0.33	0.12	0.10	0.08

The second figure in the text shows an X-ray diffraction diagram at different temperatures. Reference: 1 in English.

The paper was received for publication on 18 September 1987.

10424/6091

Etching of InP Semiconductor Material by Ablative Photodecomposition With a Direct Excimer Laser

40090120c Changchun JILIN DAXUE ZIRAN KEXUE XUEBAO [ACTA SCIENTIARUM NATURALIUM UNIVERSITATIS JILINENSIS] in Chinese No 2, 28 May 88 pp 114-115

[Article by Zhang Yushu [1728 3768 2885], Zhang Qingyou [1728 1987 3589], Ren Linfu [0117 5259 4395], and Shi Jinglong [4258 2529 7893] of the Electronics Sciences Department, Jilin University]

[Abstract] Etching of InP semiconductor material by ablative photo-decomposition (APD) with a direct excimer laser was accomplished for the first time. One of two figures shows the experimental arrangement. An XeF excimer laser was used at an operating wavelength of 308 ns, 20 ns pulse duration, and the maximum repetition frequency of 100 Hertz. The average power output was 17 watts, and the light beam cross section was 18 x 23 mm^2. As shown by the experimental results, there is a threshold value for light energy density for APD effect on InP material. Above the threshold value, the etching quality depends on light energy density; a satisfactory etching mirror surface and fringe quality (as shown in another figure) can be obtained with appropriate light energy density. The third figure shows the etching results when energy density is too high. References: 5 in English.

The authors are grateful to Wang Changshan [3769 7022 1311], Nan Yingzi [0589 5391 1311], and Wu Hailin [0702 3189 2651] of Changchun Institute of Optics and Precision Instruments, Chinese Academy of Sciences.

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10424/6091

Growth of $YBa_2Cu_3O_{7-x}$ Single Crystals

40090120d Changchun JILIN DAXUE ZIRAN KEXUE XUEBAO [ACTA SCIENTIARUM NATURALIUM UNIVERSITATIS JILINENSIS] in Chinese No 2, 28 May 88 pp 116-118

[Article by Zeng Lingwen [2582 0356 2429], Zhao Jianhua [6392 1696 5478], Wu Daiming [0702 0108 7686], Qian Zhengnan [0578 2973 2948], Wang Yizhong [3769 0001 0022], Zhang Yupu [1728 5940 2528], Lin Xiaomin [2651 2556 2404], and Wang Deyong [3769 1795 3196] of Physics Department, Jilin University; Fan Yuguo [2868 3768 0048] of Theoretical Chemistry Institute, Jilin University; and Cui Donghuan [1508 2639 3562] of First Department, Jilin Industrial University]

[Abstract] $YBa_2Cu_3O_{7-x}$ single crystals, 2 x 2 x 0.1 mm^2 in size, were prepared by using excess CuO or CuO + $BaCuO_2$ as the fusing agent under various conditions. After the crystals were annealed for 13 hours at 450°C in streaming oxygen, the onset superconducting transition temperature was typically 95°K.

Five figures show the heat flow versus temperature curves for quenched raw material and for excess quantities of $BaCuO_2$ and CuO as fluxes, the micrographs of single crystals grown with quenched raw materials, and the magnetizing rate versus temperature curve for a single crystal. References: 2 in English.

The paper was received for publication on 20 April 1988.

10424/6091

Preparation of Superconducting Thin Films of $YBa_2Cu_3O_{7-x}$ by Atomizing Deposition

40090120e Changchun JILIN DAXUE ZIRAN KEXUE XUEBAO [ACTA SCIENTIARUM NATURALIUM UNIVERSITATIS JILINENSIS] in Chinese No 2, 28 May 88 pp 119-120

[Article by Wu Daiming [0702 0108 7686], Wang Yizhong [3769 0001 0022], Qian Zhengnan [0578 2973 3948], Zeng Lingwen [2582 0356 2429], and Liang Luguang [2733 6424 0342] of the Physics Department, Jilin University]

[Abstract] Superconducting thin films of $YBa_2Cu_3O_{7-x}$ 3 to 5 micrometers thick were prepared on ZrO_2 single crystal substrates by atomizing deposition. The thin films made by appropriate annealing were textured with the c axis predominantly normal to the film plane and yield T_c (onset) = 95°K and T_c (R = 0) = 87°K.

This atomizing deposition has the advantages of simple equipment and operation, easily prepared and controlled constituents, and good repetition. In addition, the technique is adaptable in studying the effect on thin film superconductivity after Y, Ba or Cu is replaced with different elements. This advantage is unique to this particular film deposition technique.

Two figures show an X-ray diffraction diagram and resistance versus temperature curves. References: 5 in English.

The paper was received for publication on 20 April 1988.

10424/6091

Tl-Ca-Ba-Cu-O Superconductors With T > 100°K

40090120f Changchun JILIN DAXUE ZIRAN KEXUE XUEBAO [ACTA SCIENTIARUM NATURALIUM UNIVERSITATIS JILINENSIS] in Chinese No 2, 28 May 88 pp 121-122

[Article by Wu Daiming [0702 0108 7686], Qian Zhengnan [0578 2973 3948], Zeng Lingwen [2582 0356 2429], and Chen Hongqi [7115 1347 3823] of Physics Department, Jilin University; and Zhang Zhikun [1728 1807 3540] of Material Science Institute, Jilin University]

[Abstract] The paper reports on a preliminary study of a Tl-Ca-Ba-Cu-O system. By selecting the nominal composition of $Tl_2Ca_2Ba_1Cu_3O_x$, the authors obtained thallium-containing superconducting ceramics with T_c (onset) = 123°K and T_c (R = 0) = 109°K.

By applying four terminal lead wire method with a Pt resistance thermometer, resistance versus temperature curves of two specimens are shown in one of three figures in the text. Two other figures show a microscopic picture and an X-ray diffraction diagram of one specimen. One table lists data of X-ray diffraction diagram of the above-mentioned specimen. References: 4, 2 in English and 2 in Chinese.

The paper was received for publication on 20 April 1988.

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END

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